FERTILITY AND CYTOGENETICS OF *Vaccinium darrowi* CAMP x *V. arboreum* MARSH F-1 HYBRIDS AND THEIR OPEN-POLLINATED DERIVATIVES

By

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Fertility, unreduced gamete production, cytogenetics, and ability to cross with tetraploid (2n=4*x*=48) V. corymbosum L. were studied in two taxa: (1) diploid intersectional F-1 hybrids between V. darrowi Camp (2n=2*x*=24, section Cyanococcus) and V. arboreum Marsh (2n=2*x*=24, section Batodendron) and (2) seedlings, termed Mother is Known (MIK), obtained by open pollinating the F-1's in a field where pollen from section Cyanococcus species V. corymbosum (4*x*) and V. ashei Reade (6*x*) were available. Female and male fertility was very low in the diploid F-1 hybrids, but were much higher (although variable) in the MIKs. Cytogenetic analysis showed low levels of chromosome pairing at metaphase I in the F-1 hybrids and in the least-fertile MIKs, which led to lagging chromosomes at anaphase I and anaphase II and aneuploid gametes at telophase II. In the most fertile MIKs, most pairing at metaphase I was bivalent and most disjunction at anaphase I and anaphase II was normal. The more frequent bivalent

pairing in the MIKs compared to the F-1 hybrids was probably due to the fact that most of the MIK chromosomes were derived from section Cyanococcus and fewer from V. arboreum. About one quarter of the F-1 hybrid population produced some unreduced gametes, an ability possibly derived from their V. darrowi parent. Unreduced gametes allowed the F-1 diploid hybrids to produce tetraploid hybrids when pollinated with pollen from V. corymbosum, and at least one pentaploid hybrid was produced, apparently from pollination of the diploid F-1 hybrids by hexaploid V. ashei. The high frequency of univalents in most of the diploid intersectional hybrids indicates that V. darrowi and V. arboreum have diverged genetically. However, their hybrids can be used as a bridge to obtain tetraploid hybrids with V. corymbosum in which fertility is medium to high and V. arboreum genes and characteristics are present.

CHAPTER 1
INTRODUCTION

The genus Vaccinium L. is essentially worldwide in distribution, with the greatest concentration of species in the tropical mountains (Vander Kloet, 1983). The genus is also well represented in temperate North America, where species belonging to ten different sections occur. Worldwide, three Vaccinium sections contain important crops: section Cyanococcus (blueberry in the narrow sense), section Oxycoccus (cranberry) and section Vitis-idaea (lingonberry). Germplasm useful in breeding blueberries includes cultivated and non-cultivated species within section Cyanococcus and several species of Vaccinium from sections other than Cyanococcus. The cultivated blueberries consist of three species in Vaccinium section Cyanococcus. In addition to two cultivated tetraploid ($2n=4x=48$) species (V. corymbosum and V. angustifolium Ait.) and one hexaploid ($2n=6x=72$) cultivated species (V. ashei Reade), section Cyanococcus contains several diploid, tetraploid and hexaploid species that have never been domesticated. One wild diploid ($2n=2x=24$) species, V. darrowi, whose range lies almost entirely within Florida, has been crossed with V. corymbosum to produce the so-called "southern highbush" cultivars (Lyrene, personal communication).

Several attempts have been successful in producing viable intersectional F-1 hybrids in Vaccinium. However, most of these hybrids have been sterile. The ease of combining desirable traits from species in different sections of a genus depends in part on how similar the genomes of the species are.

Vaccinium arboreum Marsh, a diploid (2n=2x=24) species in section Batodendron, is potentially useful in breeding new highbush blueberry cultivars. V. arboreum traits that would be desirable in cultivated blueberry include erect growth habit, drought resistance, upland adaptation, calcareous soil tolerance, late flowering and a flower that is less dependent on sonicating bees for pollination (Brooks and Lyrene, 1995a). In 1981, experiments were initiated in the Horticultural Science Department at the University of Florida to test the possibility of using V. arboreum in breeding highbush blueberry cultivars tolerant to warm soils low in organic matter. Using V. darrowi as a genetic bridge, it was possible to obtain viable diploid V. darrowi x V. arboreum hybrids (F-1) and tetraploid derivatives, termed MIKs (Mother Is Known), from open pollination of the F-1 hybrids (Brooks and Lyrene, 1995b).

The ease of introgression of desirable traits from V. arboreum into cultivated blueberry is greatly influenced by the degree of genetic affinity among the genomes of the species involved. Thus, this study was undertaken, whose general objective was to gather information about the cytogenetics and chromosome behavior of these intersectional hybrids and their derivatives and to determine their possible usefulness in blueberry breeding.

Research objectives

The first goal of this study was to evaluate male and female fertility in two populations in which meiosis was to be studied: the F-1 V. darrowi x V. arboreum hybrids and their open pollinated derivatives (MIKs). A search was carried out to determine if the ability of V. darrowi to produce 2n-gametes was transmitted to some of the F-1 progeny from V. darrowi x V. arboreum crosses.

A second objective of this study was to analyze meiosis in V. darrowi x V. arboreum hybrids and their open-pollinated derivatives (MIKs) to determine whether there were abnormalities during meiosis that might reduce fertility and affect their usefulness as breeding lines.

A third objective was to determine if fertile hybrids could be obtained by hand pollinating V. darrowi x V. arboreum F-1 hybrids with pollen from V. corymbosum or V. ashei. In the first part of the study, some of the F-1 V. darrowi x V. arboreum hybrids produced what appeared to be unreduced (2*x*) or doubly unreduced (4*x*) gametes. Pollinations were made to determine if the frequency and type of unreduced gametes produced by an F-1 plant were correlated with the results of crossing that plant with V. ashei or V. corymbosum.

A fourth objective was to compare the fertility of V. corymbosum x V. corymbosum crosses with the fertility of V. corymbosum x MIK [(V. darrowi x V. arboreum) open pollinated] crosses to see if MIKs selected for high fertility were as male fertile as V. corymbosum cultivars when crossed with V. corymbosum. High fertility in MIKs would indicate the possibility of obtaining completely-fertile highbush blueberry plants containing genes from V. arboreum.

CHAPTER 2
REVIEW OF LITERATURE

Vaccinium arboreum MARSH

Vaccinium arboreum Marsh [section Batodendron (Nutt.) Klotztch], commonly called sparkleberry, farkleberry, tree huckleberry or winter huckleberry, is a shrub or small tree with wide distribution in the southeastern and mideastern United States. In the wild, sparkleberry occurs in sandy or rocky, usually dry woodlands of the coastal plain and piedmont from southern Virginia to Georgia and Florida, through the Gulf states, and from southern Illinois and Missouri through eastern Texas. In the south, the sparkleberry is often up to 5 meters tall with a crooked trunk, occasionally 20 to 25 cm in diameter, and has slender, more or less contorted branches which form an irregular round head. In the north, it is a shrub with many divergent stems. The bark is reddish, the flowers are white (flowering March to April, rarely in February and July) and the hard, black berries, produced in abundance in late summer (fruiting August to October), are edible but not very palatable (Camp, 1945; Allgood, 1970; and Ward, 1974).

Useful Breeding Features

Vaccinium arboreum is a drought-resistant species usually found in dry woods, thickets and clearings. Like most Ericaceous species it is most abundant on acidic soils, but it tolerates higher pH soils than most species in the family.

This broader soil adaptation suggests that sparkleberry could be a source of genes for broader soil tolerance (Ballinger et al., 1982; Lyrene, 1991; Lyrene and Brooks, 1995).

Sparkleberry flowers have characteristics that facilitate pollination by a wide range of insect species, contrasting with section Cyanococcus species, which are pollinated mostly by sonicating bees. Sparkleberry flowers have shorter corolla tubes and wider corolla openings than blueberry flowers. If these characteristics could be transferred to highbush, it could increase pollination and fruit set (Brooks and Lyrene, 1995a).

Late flowering is another sparkleberry characteristic that could be useful in blueberry breeding. Late flowering makes sparkleberry less susceptible to crop loss due to freezes than cultivated blueberries (Lyrene, 1991; Lyrene and Brooks, 1995), and would be advantageous in developing late-ripening cultivars.

Sparkleberry has several characteristics that would be undesirable in commercial cultivars. The berries are small, dark and have large seeds, abundant sclereids, and low juice content (Lyrene, 1997). Late ripening would be a disadvantage in Florida where early ripening is desired, but would be advantageous in Michigan and the Pacific Northwest where late ripening is desired.

Intersectional Crosses

According to Lyrene and Ballington (1986), gene exchange is possible among some sections in the genus Vaccinium. Sections other than Cyanococcus (blueberry) and

Oxycoccus (cranberry) that have desirable horticultural traits that could be introgressed into cultivated blueberries or cranberries, or that have potential use in development of new cultivated crops and that are represented in temperate North America include Vaccinium crassifolium and V. sempervirens (section Herpothamus); V. stamineum (section Polycodium); V. erythrocarpum (section Oxycoccoides); V. ovatum, (section Pyxothamnus); and V. arboreum (section Batodendron) (Lyrene and Ballington, 1986).

Transferring desirable traits among species in different sections of a genus depends on actual genetic affinities among the species. Several attempts have been carried out to produce viable inter-sectional hybrids in Vaccinium (Ballington et al., 1988). Ballington et al. (1987) pointed out that speciation and crossability patterns in Vaccinium might be explained by the syngameon concept, in which a large amount of genetic variation is shared among a group of species or semispecies that are not completely isolated genetically.

F-1 sterility and hybrid weakness, which separate even closely related species in many genera, appear to operate largely at the sectional level in Vaccinium (Ballington, 1990). Natural or artificial inter-sectional hybrids have been reported. These include V. uliginosum (section Vaccinium) x V. coymbosum (section Cyanococcus), whose artificial viable hybrids were obtained when V. uliginosum was used as a female parent (Rousi, 1963; Hiirsalmi, 1977; Hiirsalmi and Lehmushovi, 1982); V. myrtillus (section Myrtillus) x V. vitis-idaea (section Vitis-idaea), a naturally occurring sterile hybrid (Ahokas, 1971); V. microcarpum (section Oxycoccus) x V. vitis-idaea (section Vitis-idaea), viable hybrids from artificial reciprocal crosses (Ahokas, 1979); V. atrococcum (section Cyanococcus) x

V. stamineum (section Polycodium); V. darrowi (section Cyanococcus) x V. stamineum; V. tenellum (section Cyanococcus) x V. stamineum; V. caesariense (section Cyanococcus) x V. stamineum (Ballington, 1980); V. darrowi x V. arboreum (section Batodendron), and V. atrococcum x V. arboreum (Lyrene, 1991).

Along with Vaccinium cubense Griseb of Cuba and Hispaniola, and V. leucanthum and V. stenophyllum of Central America, V. arboreum is considered a disjunctive member of a complex of species centered in Central America and Mexico (Wood, 1961). Definite genetic affinities between Vaccinium sections Batodendron and Cyanococcus were first demonstrated when Galletta and Fish (1971) were successful in grafting highbush blueberry (V. corymbosum) on V. arboreum. Chemotaxonomy studies carried out by Ballinger et al. (1982) determined that the extracted and purified anthocyanins in fruits of V. arboreum were extremely similar to those reported for the fruits of highbush and lowbush blueberries (both in section Cyanococcus) and more different from those of V. stamineum (section Polycodium), a species that had been considered by floral morphology, ontogeny and geographical distribution to be more closely related to V. arboreum than to Cyanococcus species. However, the same authors stated that as of 1982 there was no evidence of genetic exchange between section Cyanococcus species and V. arboreum.

Another study (Goldy et al., 1984), in which pollen morphology in V. arboreum, V. stamineum, and eight species in section Cyanococcus were analyzed, determined that the tectum or outermost exine layer in the pollen grain of V. arboreum was distinctly different from that of the other species, which were somewhat similar for this character.

Vaccinium arboreum Breeding

According to Lyrene (1997) the first crosses with sparkleberry were made in 1981, when pollen from V. arboreum was used to pollinate flowers of V. darrowi. The F-1 hybrids were very variable in vigor, but morphologically they were intermediate between the parents for several traits (Brooks and Lyrene, 1995a). The F-1 hybrids were allowed to be open-pollinated in the presence of various other Vaccinium species. The most fertile of the resulting open-pollinated plants, termed MIK (mother is known), were tetraploid or near tetraploid, and were believed to have been produced when unreduced megagametes from the diploid V. darrowi x V. arboreum hybrids united with normal gametes from tetraploid V. corymbosum. These derivative hybrids were highly variable, but many were vigorous, flowered heavily and grew more vigorously on low-organic soils than southern highbush blueberry (Brooks and Lyrene, 1995b). The most fertile MIKs were selected and crossed to V. corymbosum cultivars. The progeny plants were usually more fertile than the MIKs, and, based on the amount of berries they produced, some plants appeared to be as fertile as normal highbush plants, which indicated that the combination of backcrossing and selection could produce populations that are highly fertile (Brooks and Lyrene, 1995a).

Vaccinium Genome Characteristics

In 1927, Longley described meiosis in several diploid, tetraploid and hexaploid species of Vaccinium and found regular bivalent pairing, with a few open and closed ring bivalents in some species.

The same author reported twelve bivalent chromosomes at diakinesis in the pollen mother cells of the diploid species and concluded that twelve is the basic number for the genus.

Hall and Galleta (1971), studying the mitotic morphology of several diploid species in section Cyanococcus, reported that the karyotypes were indistinguishable morphologically, small in size and symmetrical in shape. The basic haploid genome in Vaccinium consists of two long, eight intermediate and two short metacentric or submetacentric chromosomes.

Cockerham and Galletta (1976), analyzing meiosis in diploid, tetraploid and hexaploid species in Vaccinium, found that in interphase, the nucleolar number varied from 1 to 2 in each ploidy level, suggesting that nucleolar number is not a useful indicator of ploidy level in Vaccinium.

Chromosome pairing and disjunction that are more-or-less regular have been reported for many Vaccinium species, cultivars and even hybrids (Galleta and Ballington, 1996). Several authors (Jelenkovich and Hough, 1970; Newcomer, 1940; and Stushnoff and Hough, 1968) have studied meiosis in cultivated varieties of V. corymbosum, reporting essentially regular bivalent pairing, with some multivalence and pseudo-multivalence (secondary pairing), which was generally interpreted as an indication of allopolyploidy. However, because blueberry chromosomes are very small and largely indistinguishable, exact pairing relationships have not been resolved, and because pseudo-multivalents may occur, the interpretation of meiotic configurations is highly subjective, (Riley, 1960; Ahokas, 1971; Qu et al., 1998).

Cockerham and Galletta (1976) found complete synapsis and equal segregation in chromosomes during meiosis in V. elliottii (diploid), V. australe (tetraploid) and V. amoenum (hexaploid). However, half of the meiocytes studied in V. amoenum had 1 or 2 multivalents, consisting of a very close secondary association of regular bivalents, which appeared to represent secondary association of homoeologous pairs of chromosomes.

Jelenkovich and Harrington (1971), after finding only bivalent and quadrivalent associations without any univalent or trivalent associations as would be expected with random pairing of four homologous chromosomes, postulated that obligatory instead of random pairing of chromosomes occurs in tetraploid V. australe. Meiosis was normal and balanced gametes were produced. The authors concluded that an obligatory synapsis and a localized distal chiasma might account for the exclusive bivalent and quadrivalent associations in that genotype.

Jelenkovich and Hough (1970) reported secondary associations or residual attraction between distantly related chromosomes in V. corymbosum. These secondary associations were expressed at metaphase I as close associations of bivalents into groups of two, three, or four, forming pseudo-multivalents. Secondary associations were first experimentally demonstrated in hexaploid Triticum, where pseudo-quadrivalents and pseudo-hexavalents were the most frequent secondary associations (Riley, 1960). Secondary associations occur mostly in polyploids and in groups like cultivated highbush blueberry where the tetraploid cultivars are actually hybrids involving V. corymbosum and other tetraploid species. Meiotic analysis of hybrids of V. corymbosum with some of

its purported ancestral species, done by Newcomer (1940) and Rousi (1963), have revealed evidence of structural hybridity in some V. corymbosum chromosomes.

The genetic background of the tetraploid-hybrid clones that were studied by Jelenkovich and Hough (1970) varied widely, and a dissimilar pattern of chromosome behavior in meiosis was expected. However, they found that for most clones the proportion of chromosomes involved either in independent bivalents or in true quadrivalents was very low. A large portion of the total genome was involved in pseudo-multivalent associations, which were the consequence of incomplete relaxation of the synezetic knot observed at early prophase.

Numerous irregularities during meiosis in pollen mother cells have been reported in hybrids from crosses between species with different ploidy level. Goldy and Lyrene (1983) found several irregularities at meiosis in V. ashei (2n=6*x*=72) x V. darrowi (2n=2*x*=24) hybrids including 60 somatic chromosomes instead of the expected 48, two synezetic knots, two nucleolar organizing regions, lagging chromosomes at anaphase I and II, non associating chromosomes, meiotic asynchrony, micro nuclei at telophase I and II, misaligned spindles, extra nucleoli, increased percentage of unreduced gametes, incomplete tetrads and sporads with 5 or more spores.

Unreduced Gamete Production

According to Ortiz et al., (1992) there are two ways in which polyploids can arise: somatic doubling of chromosomes by endomitosis (asexual polyploidization) or by

modification of the meiotic process leading to the formation of 2n-gametes (sexual polyploidization).

Gametes with the sporophytic chromosome number are known as 2n-gametes. Such gametes are the result of a modified gametogenesis, which in some individuals is under the genetic control of meiotic mutants, but which may also be affected by the environment (Ortiz et al., 1998).

The existence and significance of 2n-gametes in blueberry (Vaccinium section Cyanococcus) are evidenced by numerous polyploid species that exist in this section. The occurrence of 2n-gametes is widespread in diploid and polyploid blueberry species (Ortiz et al., 1992). Many species in Cyanococcus produce some functional 2n-gametes. This has been demonstrated by the recovery of variable numbers of tetraploid hybrids from heteroploid 4*x*-2*x* interspecific crosses (Lyrene and Sherman, 1983).

As with 2n-pollen, functional 2n-egg formation has been demonstrated by the recovery of tetraploid progeny from (2*x*) x (4*x*) crosses and hexaploid progeny from (3*x*) x (3*x*) crosses (Vorsa and Rowland, 1997). The same authors mention several mechanisms of 2n gamete formation, including premeiotic doubling, first division restitution (FDR), chromosome replication during meiotic interphase, second division restitution (SDR), postmeiotic chromosome doubling, and apospory. Restitution in FDR and SDR refers to the formation of a single nucleus with the unreduced chromosome number as a result of failure of the first or second division in meiosis, respectively.

CHAPTER 3
FERTILITY OF V. darrowi x V. arboreum F-1 HYBRIDS AND
V. darrowi x V. arboreum OPEN-POLLINATED DERIVATIVES (MIK)

Introduction

Brooks (1996) pollinated several thousand flowers of southern highbush cultivars (tetraploid section Cyanococcus) with pollen from several different plants of V. arboreum (diploid, section Batodendron) and obtained no hybrids. In an effort to move V. arboreum genes into the southern highbush gene pool, V. darrowi, a diploid species in section Cyanococcus, was used as a genetic bridge.

Several thousand V. darrowi plants were pollinated with pollen from several different V. arboreum plants, and thousands of F-1 seedlings were obtained (Lyrene, 1991). About 200 of these were transplanted to a field nursery in 1994, and the most vigorous seedlings, whose morphology showed they were hybrids, were transplanted after two years to another field plot. It was hoped that these F-1 intersectional hybrids could be used as a genetic bridge in crosses with southern highbush cultivars.

Previous studies (Lyrene, 1995) had determined that open-pollinated progeny from the V. darrowi x V. arboreum F-1 hybrids, known as MIK (Mother is Known), are highly variable in fruitfulness after open pollination in the field. When the most fruitful MIK plants were pollinated in a greenhouse with pollen from southern highbush cultivars, they produced numerous viable progeny. Therefore, according to the same author, the fertile MIKs were probably tetraploid or near tetraploid (aneuploid).

The origin of the MIKs is not known with certainty, because their F-1 mother plants had been open pollinated in a field containing hexaploid V. ashei and various wild diploid species as well as tetraploid southern highbush selections, but it was considered most likely that they were formed by the union of an unreduced egg from the F-1 hybrid and a normal microgamete from tetraploid southern highbush (Lyrene, 1991; Lyrene and Brooks, 1995).

The objectives of this study were as follows:

1) To evaluate the male fertility of 103 V. darrowi x V. arboreum F-1 hybrids and 47 V. darrowi x V. arboreum open-pollinated derivatives of the F-1 hybrids plants (MIK) compared to the parental species. This was done by estimating the quantity of pollen shed and the percent of the pollen that could be stained with acetocarmine.

2) To evaluate female fertility by observing fruit set and seed production on each F-1 hybrid plant and each MIK plant after open pollination in the field.

3) To determine and quantify the presence of unreduced gametes in the pollen of F-1 V. darrowi x V. arboreum hybrids.

Material and Methods

The V. darrowi x V. arboreum F-1 hybrid population used in this study originally consisted of 119 plants. Some of these were weak and died during the study, so only 110 F-1 plants were studied. These F-1 hybrids originated from seven crosses, whose parents are given in Table 1. In all cases, both parents had been selected from wild populations in Florida. The V. darrowi parents were selected from Farles Prairie in the Ocala National Forest, east of Ocala, Florida, based on large fruit size, vigorous plants, and blue fruit

color. The V. arboreum plants were selected from a forest near Boulware Springs, Alachua County. All plants were propagated by softwood cuttings and were maintained at the Horticultural Unit, University of Florida, Gainesville. Crosses were made in March, 1993 using V. darrowi and V. arboreum plants that had been dug the previous December and kept in pots in a greenhouse over winter. Seeds from the crosses were sown in November, 1993. Seedlings were moved to a field high-density nursery in May, 1994. After 1 1/2 years in the field, the most vigorous 119 seedlings that showed characteristics that were intermediate between the parent species were transplanted to a 1 x 3-m spacing in January, 1996. The fertility analyses were initiated on January, 1998, when the plants were in their fifth growing season. Plants growing near the F-1 hybrids which could have served as pollen sources included (in addition to the hybrid population) diploid (V. darrowi and V. elliottii), tetraploid (V. corymbosum), and hexaploid (V. ashei).
The MIK population was formed by 49 plants that originated from open- pollinated seeds from F-1 V. darrowi x V. arboreum hybrids. The open-pollinated seeds were extracted from the F-1 hybrids, stored dry at 7°C, and germinated on Canadian peat under intermittent mist in a greenhouse. Surviving seedlings were transplanted to a high-density nursery in May, 1995. The plants were evaluated during the 1998-1999 period when they were in the fourth and fifth growing seasons.

During January, 1998 and 1999, before anthesis, flowers were collected from F-1 V. darrowi x V. arboreum hybrids, V. darrowi x V. arboreum open-pollinated derivatives (MIKs), and from V. darrowi plants maintained at the Horticultural Unit in Gainesville, Florida. Also, flowers from V. arboreum were obtained from wild plants in the forest near the Horticultural Unit in Gainesville during March 1998 and 1999.

Table 1. Parents of crosses that produced the F-1 hybrid population.

V. darrowi	x	V. arboreum[z]	Number of F-1 hybrid plants studied
91-323 Farles[y]		93-2	16
91-323 Farles		Boulware 2	17
91-318 Farles		93-2	7
91-317 Farles		93-2	22
91-323 Farles		93-1	44
91-323 Farles		Boulware 4	3
91-331 Farles		Boulware 4	1

[z]Clone 93-1 and 93-2 were collected from Gainesville, Florida, Boulware 2 and 4 were collected from Boulware Springs, Florida.
[y]Clones from Farles Pairie, Ocala National Forest, Ocala, Florida.

Flowers were collected and stored in paper bags until their examination. To determine quantity of pollen, the end of the corolla tube of five flowers was opened by cutting it with a scissors, and the pollen was removed by gently rolling the flowers between the thumb and index finger over a microscope slide. Each clone was rated for the amount of pollen shed using a 9-point scale that ranged from 1 representing no pollen to 9 representing a copious amount of pollen. The reading of "9" was set as equal to the amount of pollen that could be obtained from the most copious pollen shedders among the southern highbush cultivars.

To determine potential pollen viability, a drop of the 2 % acetocarmine-jelly (Radford et al., 1974) was placed on a microscope slide, and the pollen from five flowers was mixed into the drop and examined at 250x using a Leitz light field microscope.

After the pollen had been in the dye for at least 15 minutes, four hundred pollen grains per clone were scored for staining, and the data were expressed in percentage

stainability. Pollen grains that were plump and well stained were scored as viable. Grains that were granular, not stained, poorly stained, or shrunken were scored as non-viable.

In addition, four hundred pollen grains were counted, including stained and unstained, reduced and unreduced, to determine the frequency of stained unreduced gametes. Pollen was considered to be unreduced if it was significantly larger in diameter than the average for the species and occurred in a diad or monad instead of the usual tetrad of microspores (Cockerham and Galletta, 1976). Only well-stained pollen was considered in calculating unreduced gamete frequency, and the frequency of unreduced gametes was estimated using the equation (A+2B)/T where A is the number of monads, B is the number of diads, and T is the total number of pollen grains examined (Dweikat and Lyrene, 1988). However, when stained, unreduced pollen grains could not be found, unstained, unreduced gametes were recorded as a reference.

Tetrad (4 pollen grains attached together) spore diameter was measured at the widest point of the tetrad using only those tetrads which were well stained and apparently fertile. Diads were measured at the union region of the two pollen grains. When stained tetrads could not be found, non-stained tetrads were measured.

In the years 1998 and 2000, the relative abundance of flowers and fruit produced on F-1 and MIK plants after open pollination in the field was estimated using a 9-point scale that ranged from 1 representing no flowers (fruit) to 9 representing a high number of flowers (fruit) per plant. On plants that made berries, seeds were extracted manually and the number of seeds per berry was determined for ten berries during the ripening period. Both number of plump seeds and total number of seeds were recorded, but only the plump seeds were considered viable.

Statistical Analysis

Four variables were measured in all the F-1 plants for two years: flower abundance and fruit production (Table 2) and amount of pollen shed per flower and percent pollen staining (Table 3). A randomized block ANOVA was conducted to determine whether there were significant differences among clones. An F test, which was calculated as Mean Squares for clones divided by Means Squares for clone x year interaction, was the basis for this test.

The F-1 V. darrowi x V. arboreum plants were derived from 7 different crosses involving 4 different V. darrowi clones and 4 V. arboreum clones. Only one F-1 plant was studied for one of the crosses. In the other crosses, the number of F-1 plants studied ranged from 2 to 44 plants. Mean values for all the variables that were measured were calculated for each cross.

To determine whether the crosses differed significantly for any of the variables, a completely random analysis of variance was made in which the variability among crosses was compared with the variability among plants within crosses using an F test. Before the analysis, the data for pollen stainability percentage were modified by using the angular or arcsine transformation (Steel and Torrie, 1960).

Results and Discussion

F-1 Hybrid Population

From an original F-1 V. darrowi x V arboreum population of 119 plants, two plants were found not to be intersectional hybrids. Indications that they were not hybrids

included lack of distinctive V. arboreum characteristics and high pollen fertility. They are believed to have resulted from self-pollination of the V. darrowi parent. Seven other plants died during the period of evaluation. The other 110 plants showed high variability in vigor and plant size. During fall 2000, after 7 growth seasons, the average height was 103.0 cm and the average canopy diameter was 81.7 cm. Canopy diameter ranged from 38 to 175 cm and height from 12 cm to 137 cm, respectively (Table 2). According to height and canopy diameter data, 25.5 % of the plants were classified as large, 42.2 % as medium and 32.3 % as small.

High variability among clones was also observed in the amount of flowers produced (Table 2). Six clones (5.9%) did not flower either year. Nine other clones (8.8%) flowered one year but not the other. All other clones flowered both years. The number of flowers produced per plant varied from abundant (similar to prolific plants of the parental species) to very few.

The amount of flowers was independent of the vigor and size of the plant. Some small plants produced numerous flowers and some large vigorous plants did not flower. It was not possible to determine if those vigorous plants were genetically incapable of flowering or whether they only needed more years to reach the age of flowering.

The amount of berries produced per plant was very low compared to the number of flowers observed. Sixty-two clones (60.8 %) produced no fruit at all in two seasons that were evaluated. In the 1998 harvest, only 8 clones produced more than 100 berries per plant even though the most floriferous plants had produced several thousand flowers each. Berry number per plant ranged from 111 to 350 berries for these 8 clones (data not shown). The number of plump seeds per berry, as revealed by 10-berry samples per bush,

Table 2. Plant size, flower abundance, berry production and seed number per berry of F-1 progeny from seven crosses between Vaccinium darrowi (2n=2x=24) and V. arboreum (2n=2x=24).

Pedigree/ clone	Plant height[z] (cm)	Plant diameter[z,y] (cm)	Flower production[x] (1-9 scale)	Fruit productio[w] (1-9 scale)	Number of plump seeds per fruit[v]
91-323 Farles V. darrowi x 93-2 V. arboreum					
98-49	86	98	6.0	1.0	0.0
98-50	168	119	6.0	2.8	0.5
98-51	112	103	6.0	1.0	--[u]
98-52	114	104	7.6	1.0	--
98-53	171	119	5.9	1.0	--
98-54	122	85	5.5	1.0	--
98-55	102	111	5.2	1.0	--
98-56	117	89	1.9	1.0	--
98-57	127	133	8.7	1.1	0.0
98-58	127	83	4.2	1.0	--
98-59	91	93	6.0	1.0	--
98-60	160	113	5.4	1.1	0.1
98-61	150	118	5.4	1.1	0.5
98-62	64	62	4.8	1.0	--
98-63	125	114	6.2	1.1	0.0
98-64	107	90	4.2	1.0	--
91-323 Farles V. darrowi x Bulware 2 V. arboreum					
98-66	130	55	4.8	1.0	--
98-67	145	78	4.7	3.5	0.1
98-68	109	84	5.1	1.3	0.1
98-69	99	72	4.9	3.5	0.2
98-70	147	137	4.7	1.8	0.1
98-71	89	57	2.8	1.0	--
98-72	127	112	4.4	1.5	0.0
98-73	158	65	4.0	1.1	0.0
98-74	127	88	1.8	1.0	--
98-75	119	94	4.4	1.5	0.1
98-76	175	114	3.5	1.5	0.1
98-77	109	85	1.9	1.0	--[u]
98-78	122	76	4.6	1.0	--
98-79	46	32	1.0	1.0	--
98-80	59	25	1.4	1.0	--
98-81	79	69	3.7	1.5	0.0
98-82	114	69	3.2	1.5	0.2

Table 2. Continued.

Pedigree/ clone	Plant height[z] (cm)	Plant diameter[z,y] (cm)	Flower production[x] (1-9 scale)	Fruit production[w] (1-9 scale)	Number of plump seeds per fruit[v]
91-318 Farles V. darrowi x 93-2 V. arboreum					
98-83	38	60	1.9	1.0	--
98-84	104	95	6.5	1.5	na[t]
98-85	46	41	1.5	1.0	--
98-86	53	37	1.0	1.0	--
98-87	56	38	1.4	1.0	--
98-90	81	61	4.2	1.0	--
98-91	71	46	2.5	1.0	--
91-317 Farles V. darrowi x 93-2 V. arboreum					
98-92	135	123	8.8	1.0	--
98-93	140	12	7.5	1.1	0.1
98-94	150	119	5.7	1.0	--
98-95	137	109	5.4	1.3	0.2
98-96	91	91	5.9	1.0	--
98-97	89	76	4.2	1.0	--
98-98	74	76	1.8	1.0	--
98-99	58	64	4.4	1.3	0.0
98-101	48	42	2.1	1.0	--
98-103	48	55	2.1	1.0	--
98-104	91	78	5.8	1.3	0.0
98-105	66	33	1.0	1.0	--
98-107	76	69	6.6	1.8	0.0
98-108	64	64	1.8	1.0	--
98-109	66	61	1.0	1.0	--
98-110	na	na	2.4	1.8	0.0
98-111	61	66	5.3	1.8	na[t]
98-112	69	74	1.0	1.0	--
98-113	79	71	2.9	1.0	--
98-114	102	53	5.4	1.0	--
98-115	145	130	6.1	1.0	--
98-116	114	99	6.2	1.0	--
91-323 Farles V. darrowi x 93-1 V. arboreum					
98-117	127	83	7.4	3.0	0.5
98-118	91	66	5.3	1.0	--
98-119	97	93	5.9	1.1	0.5
98-120	137	128	6.8	1.1	0.0

Table 2.Continued.

Pedigree/ clone	Plant height[z] (cm)	Plant diameter[z,y] (cm)	Flower production[x] (1-9 scale)	Fruit production[w] (1-9 scale)	Number of plump seeds per fruit[v]
91-323 Farles V. darrowi x 93-1 V. arboreum (Continued)					
98-121	84	81	4.8	1.0	--[t]
98-122	122	99	4.7	1.0	--
98-123	178	118	5.9	2.1	0.2
98-124	102	98	4.6	1.0	--
98-125	84	103	5.6	1.0	--
98-127	135	113	5.9	1.0	--
98-128	189	102	7.7	2.8	0.7
98-129	43	34	1.4	1.0	--
98-130	122	109	6.2	1.0	--
98-131	107	85	5.3	1.0	--
98-132	155	107	6.3	1.0	--
98-133	64	37	1.8	1.0	--
98-134	86	61	4.6	1.0	--
98-135	127	103	4.6	1.5	na[t]
98-136	66	56	4.7	1.0	--
98-137	74	80	4.2	1.0	--
98-138	99	78	3.8	1.0	--
98-139	97	69	4.7	2.0	0.4
98-140	137	100	5.7	1.1	0.8
98-141	na	na	2.2	1.2	0.7
98-142	144	117	4.9	1.5	0.3
98-143	81	65	3.3	1.0	--
98-144	104	85	3.5	1.5	0.4
98-145	165	123	7.5	1.4	na
98-146	158	131	5.9	1.0	--
98-147	64	43	3.8	1.0	--
98-148	53	58	3.0	1.0	--
98-149	99	99	3.7	1.0	--
98-150	79	86	5.3	1.1	0.1
98-151	69	74	3.8	1.5	0.3
98-152	na	na	3.1	1.0	--
98-153	81	56	1.9	1.0	--
98-154	na	na	3.5	1.0	--
98-155	81	62	2.2	1.2	--
98-156	67	66	3.4	1.0	--
98-157	na	na	3.9	1.1	0.3
98-158	na	na	2.4	1.0	--
98-159	na	na	3.3	1.2	0.8

Table 2. Continued.

Pedigree/ clone	Plant height[z] (cm)	Plant diameter[z,y] (cm)	Flower production[x] (1-9 scale)	Fruit production[w] (1-9 scale)	Number of plump seeds per fruit[v]
91-323 Farles V. darrowi x 93-1 V. arboreum (Continued)					
98-160	na	na	2.4	1.0	--
98-161	107	81	4.4	1.1	0.3
91-323 Farles V. darrowi x Bulware 4 V. arboreum					
98-162	na[t]	na	5.1	2.0	0.4
98-163	61	56	5.1	1.0	--[u]
98-165	132	103	7.5	2.1	0.2
91-331 Farles V. darrawi x Bulware 4 V. arboreum					
98-167	81	53	4.7	1.0	--
Probability[s]			P<0.001	P<0.005	N.A[v]

[z] Fall, 2000. Plants had been growing in this location for 5 years.
[y] Canopy diameter: average of east-west measurement with north-south measurement.
[x] Mean of two springs (1998+2000). Scale (1-3) = low, (4-6) medium, (7-9) = high; 1 = zero, 9 = maximum.
[w] Mean of two Summers (1998+2000). Scale (1-3) =low, (4-6) =medium, (7-9) =high; 1=zero, 9 = maximum.
[v] Summer 1998, Mean of 10 berries. Fruit was not available for all the clones, therefore data were not analyzed statistically.
[u] Clones that did not bear fruit in 1998.
[t] Data not available.
[s] Probability that there were not significant differences among clones in a randomized block analysis of variance with 103 clones and two years.

was very low. From 42 clones that yielded berries, 10 produced no plump seed. The reminder clones averaged less than one seed per berry (Table 2).

Almost all clones that flowered produced some pollen (Table 3). The amount of pollen shed was variable among clones and was low compared to that of the parental species (Table 4). From 104 clones evaluated for abundance of pollen shed, only 15 were

Table 3. Pollen abundance, pollen staining, pollen tetrad diameter and 2n-gamete frequency of F-1 progeny from five crosses between Vaccinium darrowi (2n=2*x*=24) and V. arboreum (2n=2*x*=24).

Pedigree/ clone	Pollen amount[z] (1-9 scale)	Stainable pollen[zx] (%)	pollen tetrad diameter[y] (microns)	2n-gamete frequency[w] spring 1998 (%)	2n-gamete frequency[w] spring 1999 (%)
91-323 Farles V. darrowi x 93-2 V. arboreum					
98-49	3.5	2.5	(32.4)	--[u]	--
98-50	3.6	26.2	(43.9)	23.0	29.3
98-51	2.6	0.1	(30.2)	--	--
98-52	3.4	7.9	41.0	3.6	1.2
98-53	4.0	0.2	35.3	--	--
98-54	4.7	11.2	43.2	1.3	0.7
98-55	5.4	6.3	47.5	8.5	1.9
98-56	2.2	0.0	(29.5)	--	--
98-57	5.3	3.5	(33.8)	--	--
98-58	2.4	4.3	36.0	4.4	0.5
98-59	3.6	0.1	(33.1)	--	--
98-60	4.7	9.3	(33.8)	6.3	9.1
98-61	3.5	1.4	32.4	--	--
98-62	3.8	1.4	(34.5)	--	--
98-63	4.4	0.7	33.1	(2.4)	--
98-64	3.4	0.0	(31.8)	--	--
91-323 Farles V. darrowi x Bulware 2 V. arboreum					
98-66	4.3	0.4	(34.5)	--	--
98-67	4.2	0.0	(30.2)	(0.5)	--
98-68	1.4	0.0	(32.4)	--	--
98-69	4.5	1.6	36.0	--	--
98-70	4.4	1.5	(34.2)	1.0	1.6
98-71	2.3	0.6	(30.6)	--	1.0
98-72	2.4	14.7	39.6	1.0	3.9
98-73	2.9	0.0	(34.8)	--	--
98-75	1.6	0.2	(36.6)	--	--
98-76	3.4	0.7	32.4	--[u]	--
98-77	4.3	0.7	33.0	--	--
98-78	3.5	0.5	34.5	--	--
98-80	5.4	5.0	35.3	--	--
98-81	2.4	0.0	(30.6)	--	--
98-82	3.8	5.8	48.2	(6.5)	2.2

Table 3. Continued.

Pedigree/ clone	Pollen amount[z] (1-9 scale)	Stainable pollen[zx] (%)	pollen tetrad diameter[y] (microns)	2n-gamete frequency[w] spring 1998 (%)	spring 1999 (%)
91-318 Farles V. darrowi x 93-2 V. arboreum					
98-83	4.9	4.2	(36.7)	--	--
98-84	4.7	12.8	34.5	--	--
91-317 Farles V. darrowi x 93-2 V. arboreum					
98-90	7.4	6.0	38.5	--	--
98-91	6.0	4.4	35.3	--	--
98-92	4.4	9.5	38.2	--	--
98-93	4.2	4.3	33.1	--	--
98-94	4.0	5.8	37.4	--	--
98-95	5.2	17.9	42.0	--	--
98-96	3.8	1.8	35.4	--	--
98-97	4.7	10.9	49.5	--	1.3
98-98	3.4	4.9	31.7	--	--
98-99	2.3	5.7	--	5.7	--
98-103	5.2	9.5	38.4	0.9	0.5
98-104	3.7	7.4	40.2	1.9	0.2
98-105	6.7	16.8	41.4	--[u]	--
98-107	5.0	13.1		--	--
98-110	1.4	1.4	--	--	--
98-111	4.7	1.7	36.0	--	1.0
98-112	5.6	14.0	39.6	--	--
98-114	3.3	16.0	45.0	--	--
98-115	4.1	1.6	34.6	--	--
98-116	4.2	5.3	36.6	--	--
91-323 Farles V. darrowi x 93-1 V. arboreum					
98-117	4.3	0.4	30.0	--	--
98-118	4.5	1.2	35.7	--	--
98-119	3.4	0.0	(32.1)	--	--
98-120	3.5	0.0	(30.6)	--	--
98-121	4.0	0.1	(30.9)	--	--
98-122	4.3	0.0	(29.5)	--	--
98-123	4.1	2.7	31.7	--	--
98-124	4.2	4.1	33.8	--	--
98-125	4.2	23.9	41.0	--	7.9
98-127	4.1	1.0	41.4	--	--

Table 3. Continued.

Pedigree/ clone	Pollen amount[z] (1-9 scale)	Stainable pollen[zx] (%)	pollen tetrad diameter[y] (microns)	2n-gamete frequency[w] spring 1998 (%)	spring 1999 (%)
91-323 Farles V. darrowi x 93-1 V. arboreum (Continued)					
98-128	5.4	2.0	38.4	0.9	--
98-129	2.1	2.0	na[t]	1.0	na
98-130	4.6	0.2	(28.1)	--	--
98-131	3.0	0.0	(25.9)	--	--
98-132	4.5	1.5	36.0	--	1.0
98-133	1.5	13.2	37.8	--	--
98-134	4.8	3.8	46.2	1.0	0.9
98-135	4.6	4.4	36.7	--[u]	--
98-136	1.4	0.0	(29.5)	--	--
98-137	3.3	0.0	(30.2)	--	--
98-138	4.0	0.0	(32.4)	--	--
98-139	5.6	4.4	42.5	6.1	--
98-140	5.1	2.4	36.7	--	--
98-141	1.0	0.0	na[t]	--	--
98-142	4.6	4.9	41.0	3.3	3.1
98-143	5.7	0.5	(31.5)	--	--
98-144	2.2	0.0	(30.0)	--	--
98-145	4.1	0.0	(30.2)	--	--
98-146	5.5	1.6	33.5	1.0	--
98-147	6.4	1.6	36.9	--	--
98-148	3.9	0.7	(32.1)	--	--
98-149	4.9	0.6	(32.1)	--	--
98-150	1.3	0.0	na	(4.8)	--
98-151	1.4	0.0	na	--	--
98-152	1.4	0.0	(29.4)	(0.5)	--
98-153	3.2	1.9	36.6	--	--
98-154	1.1	0.0	na	--	--
98-155	4.6	0.4	32.7	(4.8)	--
98-156	2.5	8.3	na	--	--
98-157	4.9	0.0	(31.5)	--	--
98-158	3.4	1.5	na	1.5	--
98-159	2.1	3.4	na	--	--
98-160	3.1	0.0	(30.3)	--	--
98-161	2.8	1.0	32.4	--	--
91-323 Farles V. darrowi x Bulware 4 V. arboreum					
98-162	2.7	0.2	na[t]	--[u]	--

Table 3. Continued.

Pedigree/ clone	Pollen amount[z] (1-9 scale)	Stainable pollen[zx] (%)	pollen tetrad diameter[y] (microns)	2n-gamete frequency[w] spring 1998 (%)	2n-gamete frequency[w] spring 1999 (%)
91-323 Farles V. darrowi x Bulware 4 V. arboreum (Continued)					
98-163	2.5	3.3	45.7	--	--
98-165	4.1	1.0	32.9	--	--
91-331 Farles V. darrowi x Bulware 4 V. arboreum					
98-166	1.4	0.5	na	--	--
98-167	3.9	4.0	38.5	2.4	1.4
Probability[v]	N. S.	N.S.	--	--	--

[z] Mean of two springs (1998-1999). 9=abundance equal to that of the most fertile southern highbush blueberry cultivars; 1= no pollen shed.
[x] Actual data. Analysis of variance was calculated on transformed data by square root of Arcsine (%).
[y] Spring, 1999. Data from stained tetrads consisting of 4 attached pollen grains. Where stained tetrads were not found, unstained tetrads were measured and the data are included between brackets.
[w] Data include monads (M) and /or diads (D) calculated as ((M+2D)/Total pollen grains)x 100. Four hundred pollen grains were counted, including stained and unstained, reduced and unreduced. The percentage indicates the frequency of stained unreduced gametes in the sample of 400 total pollen grains. Where stained unreduced gametes were not found, unstained unreduced grains were counted and the data are included in parenthesis.
[u] Clones in which no monads or diads were found.
[v] Probability that there were not significant differences among clones in a randomized block analysis of variance with 103 clones and two years.
[l] Data not available

rated 5 or higher on the scale. A score of 5 on the scale was intermediate and was well below the 8 to 9 scores of the V. darrowi and V. arboreum clones (Table 4). In some clones, flowers shed no visible pollen, and it was necessary to squash the anthers in acetic acid and view them under a microscope at 250x to see if the clone produced any pollen grains. The ability of pollen from the F-1 hybrids to stain with aceto-carmine was very low compared to the parental species and to the cultivated highbush blueberry (Table 3). Twenty-three clones (22.1 %) produced no pollen that stained, and 77 clones (74 %) had

Table 4. Pollen abundance, pollen staining percent, pollen tetrad diameter, and 2n-gamete frequency of parental species Vaccinium darrowi (2n=2x=24) and V. arboreum (2n=2x=24).

Pedigree/ Clone	Pollen amount[z] (1-9 scale)	Stainable pollen (%)	Pollen tetrad diameter[y] (microns)	2n-gamete frequency[x] (%)
V. darrowi[wv]				
91-313	8.3	72.5	46.6	1.5
91-323a	8.4	79.4	43.6	0.0
91-323b	7.9	78.0	41.2	0.0
Juniper	8.5	47.7	42.5	1.4
91-318	8.6	84.7	41.4	0.0
Average	8.3	72.5	43.1	--
V. arboreum[wu]				
98-190	8.2	97.3	45.7	0.0
98-195	8.3	96.1	41.2	0.0
98-201	8.2	85.1	43.4	0.0
98-202	8.5	95.7	42.8	0.0
Golden Height 12	8.2	92.0	42.3	0.0
Average	8.3	93.2	43.1	--

[z]Mean of five flowers. Scale (1-3) = low, (4-6) = medium, (7-9) = high; 1 = no pollen, 9 = abundant pollen, equivalent to highly fertile southern highbush cultivars.
[y]Diameter of the tetrad consisting of 4 attached, stained pollen grains.
[x]Includes monads (M) and diads (D): ((M+2D)/Total pollen grain)x100. Based on a sample of 400 pollen grains.
[w]Sample from spring 1999.
[v]Allclones from Ocala National Forest, Ocala, Florida.
[u]All clones from Alachua County, Florida.

less than 5 % staining. The two clones with the highest percent pollen-staining were 98-50 and 98-125 with 26.2 and 23.9 % staining, respectively. In general, the stained pollen from the F-1 hybrids stained less intensely than pollen of the parental V. darrowi and V. arboreum species, and many of the stained pollen grains were part of tetrads that contain one to three non-stained or aborted pollen grains.

Pollen tetrad diameter was more uniform than the other pollen characteristics analyzed (Table 3). However, average pollen tetrad diameter was smaller for the F-1 hybrids than for the parental species. The average diameter for four pollen grains united in a tetrad in the F-1 hybrids was 37.7 microns (measured only in stained pollen tetrads), whereas the same measurement for V. darrowi and V. arboreum was 43.5 and 43.1 microns, respectively (Table 5).

High variability in plant vigor and flower production and very low fruitfulness and male fertility characterized the F-1 hybrid population. Similar results were found by

Table 5. Fruit production, seeds per berry, pollen abundance, pollen staining, and size of pollen tetrad in parental species Vaccinium darrowi (2n=2x=24) and V. arboreum (2n=2x=24), their F-1 hybrid and MIK derivatives.

Pedigree/ clone	Number of plants evaluated[z]	Fruit yield[x] (1-9 scale)	Plump seeds per berry[y]	Pollen amount[x] (1-9 scale)	Stainable pollen (%)	Tetrad diameter[w] (microns)	Number of plants with 2n-gametes[t]
V. darrowi[v]	5	--	--	8.3	72.5	43.1	2/4
V. arboreum[v]	5	--	--	8.3	93.2	43.1	0/5
F-1 hybrids[u]	103	1.2	0.2	3.8	3.8	37.7	25/102
MIK[u]	47	3.2	5.2	5.9	60.3	51.7	0/41
Probability				P<0.005	P<0.01	P<0.005	

[z]The number of plants evaluated in F-1 hybrids and MIKs was variable depending upon number of plants that did not yield flowers or berries.
[x]Scale (1-3) = low, (4-6) = medium, (7-9) = high; 1 = no pollen or no fruits
[y]Mean of ten fruits.
[w]Diameter based in the measure of 10 tetrads which consisting of 4 attached, stained pollen grains.
[v]Sample from one year (1999).
[u]Mean of two years (1998-1999), except for plump seeds per berry (1 year)
[t]Plant was considered to make 2n-gametes if one or more 2n-gametes were seen in a sample of 400 pollen grains. The first number indicates the number of plants with 2n-gametes. The second number indicates the number of plants evaluated for this parameter.

Lyrene (1991) and Brooks (1996) who analyzed a smaller V. darrowi x V. arboreum F-1 hybrid population. The authors found that most of the intersectional hybrids were vigorous and flowered strongly during several years of evaluation, but only a small proportion of the flowers yielded fruit and most of the fruit that matured contained either zero or one plump seed. This behavior has been observed before when Vaccinium species, belonging to different sections have been crossed. Lyrene and Ballington (1986) reported that intersectional crosses involving Vaccinium species from sections Cyanococcus, Polycodium, Herpothamnus, Bracteata, Vitis-idaea and Pyxothamnus produced viable and vigorous F-1 hybrids, but they were mainly sterile or partially fertile.

Genetic and ecological factors could explain the low fertility of the F-1 hybrids. Disharmonies between the parental genomes or between the genome of one parent and the cytoplasm of the other could cause sterility.

Other factors could have contributed to the low fruit set and seed production of the F-1 plants. The blooming period was very extended, from early January to mid-February, and many of the early flowers were killed by freezes, which reduced fruit set. Pollen from diploid, tetraploid and hexaploid species of Vaccinium growing nearby was available during the first half of the flowering period, but during the second half of flowering the pollen availability from potential pollen sources decreased (Lyrene and Brooks, 1995).

The flowering peak in the F-1 hybrids occurred in March, when most other sources of pollen had finished flowering. Self-fertility in the F-1 hybrids has not been evaluated, but both V. arboreum and V. darrowi exhibit very low self-fertility and self-

fruitfulness (Brooks and Lyrene, 1998) and self-incompatibility might also be expected in their progeny.

Of 102 F-1 hybrids clones analyzed, 25 produced some stainable 2n-gametes (Table 3). For thirteen clones, stained 2n-gametes were found in the pollen samples in both years. For the other 12 clones, stained 2n-gametes were noted in the pollen samples for only one of the two years analyzed. Another 5 clones produced 2n-gametes, but the pollen grains were unstained, and they were produced in only one of the two years analyzed. Among those clones for which 2-n gametes were seen, the frequency of 2n-gametes was variable, ranging from 0.2 to 29.3 %; with an average of 7.4 % in 1998 and 3.6 % in 1999. Monads were the most frequent type of 2n-gametes in the two years analyzed (Table 6). Of all 2n-gametes produced during 1998, 62.5 % were monads and 35.5 % were diads, whereas in 1999, 79.9 % were monads and 20.5 % were diads. During 1998, 8 clones yielded both monads and diads, 4 clones yielded only monads, and 4 clones yielded only diads. In 1999, 8 clones yielded monads and diads, 7 clones only monads, and 3 clones only diads. In some clones (e.g., 98-50) all the pollen grains that stained were 2n-gametes; normal stained pollen grains were not found.

The size of the 2n-gametes varied depending on the type of gametophyte (Table 6). Monads showed higher variability in size than diads. Stained monads ranged from 46.8 to 164.4 microns in diameter with an average of 99.1 microns, whereas stained diads ranged from 40.8 to 57.6 microns with average of 48.6.

The diameter in diads was measured along the line that separated the two pollen grains, which is shorter than the polar measure. The average diameter of monads and diads was 2.6 and 1.4 times higher than the average diameter of normal pollen grains that

Table 6. Frequency, type and size of 2n-gametes of F- 1 progeny from six crosses between Vaccinium darrowi (2n=2x=24) and V. arboreum (2n=2x=24).

Pedigree/ clone	Year 1998 frequency (%)		Year 1999 frequency (%)		Sporad diameter[z] year 1999 (microns)	
	Monads	Diads	Monads	Diads	Monads	Diads
91-323 Farles V. darrowi x 93-2 V. arboreum						
98-50	17.7	5.3	29.3	0.0	69.6	--
98-52	2.2	1.4	1.2	0.0	65.4	--
98-54	0.0	1.3	0.7	0.0	90.0	--
98-55	5.9	2.6	1.9	0.0	70.6	--
98-58	2.9	1.5	0.5	0.0	103.2	--
98-60	6.3	0.0	7.3	1.8	46.8	40.8
98-63	(2.4)[x]	0.0	0.0	0.0	--	--
91-323 Farles V. darrowi x Bulware 2 V. arboreum						
98-67	(0.5)	0.0	0.0	0.0	--	--
98-70	1.0	0.0	1.2	0.4	73.5	50.4
98-71	0.0	0.0	1.0	0.0	105.3	--
98-72	1.0.	0.0	2.6	1.3	82.4	57.6
98-82	(3.7)	2.8	1.7	0.4	86.9	54.0
91-317 Farles V. darrowi x 93-2 V. arboreum						
98-97	0.0	0.0	0.0	1.3	--	51.6
98-99	1.0	4.7	0.0	0.0	--	--
98-103	0.0	0.9	0.0	0.5	--	46.8
98-104	0.0	1.9	0.2	0.0	46.8	--
98-111	0.0	0.0	0.5	0.5	164.4	46.8
91-323 Farles V. darrowi x 93-1 V. arboreum						
98-125	0.0	0.0	6.0	2.0	57.0	57.6
98-128	0.9	0.0	0.0	0.0	--	--
98-129	1.0	0.0	0.0	0.0	--	--
98-132	1.0	0.0	0.0	0.0	--	--
98-134	1.0	0.0	0.0	1.0	--	--
98-139	5.2	1.0	0.0	0.0	--	--
98-142	0.5	2.8	1.2	1.4	120.3	48.0
98-146	0.0	1.0	0.0	0.0	--	--
91-323 farles V. darrowi x 93-1 V. arboreum						
98-150	(0.5)[x]	0.0	0.0	0.0	--	--
98-152	(4.8)	0.0	0.0	0.0	--	--

Table 6. Continued.

Pedigree/ clone	Year 1998 frequency (%)		Year 1999 frequency (%)		Sporad diameter[z] year 1999 (microns)	
	Monads	Diads	Monads	Diads	Monads	Diads
91-323 farles V. darrowi x 93-1 V. arboreum						
98-155	(3.8)	(1.0)	0.0	0.0	--	--
98-158	0.5	1.0	0.0	0.0	--	--
91-331 Farles V. darrowi x Bulware 4 V. arboreum						
98-167	1.4	1.0	0.9	0.5	154.1	43.2

[z]Monads: diameter from a single pollen grain. Diads: diameter at the union of two pollen grains.
[x]Data in parenthesis are from non-stained pollen.

were in tetrads. The difference in diameter between monads and normal tetrads was remarkable and its cause could not be found. In the analysis of 400-grain pollen samples from plants of the parental species, V. darrowi and V. arboreum, (five clones from each species), 2n-gametes were found only in two clones of V. darrowi (Table 4). The frequencies of 2n-gametes in those clones were relatively low with respect to that found in the F-1 hybrids. All unreduced gametes were diads, in contrast with F-1 hybrids, which showed both monads and diads. Parameters that measure female and male fertility for six crosses between V. darrowi and V. arboreum are shown in tables 7 and 8. There were no statistically significant differences between crosses in fruit yield and number of plump seeds per berry. Also, analysis of variance detected no difference among crosses in pollen abundance, pollen stainability or pollen tetrad size (Table 8). The high variability within crosses of the parameters evaluated and the highly uneven sample size prevented detection of any small differences that may have existed among crosses.

Table 7. Plant vigor, flower and fruit production and number of seeds per berry of progeny from six crosses between Vaccinium darrowi (2n=2*x*=24) and V. arboreum (2n=2*x*=24).

Cross		Number of plants evaluated	Plant vigor[z] (1-9 Scale)	Flower yield[y] (1-9 scale)	Fruit yield[y] (1-9 scale)	Plump seeds per berry[w]
V. darrowi	V. arboreum					
91-323 Farles	93-2	16	5.7	5.6	1.1	0.18
91-323 Farles	Bulware 2	17	5.4	3.6	1.7	0.08
91-318 Farles	93-2	7	3.1	2.7	1.1	--[t]
91-317 Farles	93-2	21	4.6	4.3	1.1	0.06
91-323 Farles	93-1	39	5.3	4.6	1.3	0.36
91-323 Farles	Bulware 4	2	4.5	6.3	1.5	0.20
Significance			P<0.025	P<0.001	N.S.	N.S.

[z]Summer (2000). Vigor scale (1-3) = low, (4-6) = medium, (7-9) = high.
[y]Mean of two year (1998-2000)
[w]Summer 1998. Based on 10 berries per clone.
[v]Significance of differences among crosses was found using analysis of variance on a randomized complete block design. Plant vigor and plump seed per fruit was analyzed by analysis of variance with unequal replication.
[t]Cross whose clones did not yield fruit; it was excluded from the analysis.

F-1 Open-Pollinated Derivative Population (MIK)

The population obtained by open pollination of V. darrowi x V. arboreum F-1 hybrids was highly variable. Plant growth and vigor varied from very strong plants to very weak ones, some of which died during the evaluation period. The plants were under stress because they had been planted in a15 x 45 cm spacing, and had become severely crowded as they grew. After 5 years of growth, the weaker seedlings had been shaded and weakened by the more vigorous plants. Flowering was not recorded in detail, but was consistently abundant in all clones except for six weakened plants that never flowered.

Table 8. Pollen analysis of progeny from six crosses between Vaccinium darrowi (2n=2x=24) and V. arboreum (2n=2x=24).

Cross		Number of plants evaluated	Pollen amount[x] (1-9 scale)	Stainable pollen[x] (%)	Tetrad size[y] (microns)
V. darrowi	V. arboreum				
91-323 Farles	93-2	16	3.8	4.7	38.4
91-323 Farles	Bulware 2	17	3.2	2.0	37.0
91-318 Farles	93-2	7	4.8	8.5	35.6
91-317 Farles	93-2	21	4.1	8.1	38.4
91-323 Farles	93-1	39	4.1	2.5	36.9
91-323 Farles	Bulware 4	2	3.3	2.2	39.3
Significance[u]			N.S.	N.S.	N.S.

[x]Mean of two years(1998-1999). Scale (1-3) = low, (4-6) = medium, (7-9)= high; 1 = no pollen, 9 = abundant pollen, equivalent to highly fertile southern highbush cultivars.
[y]Spring, 1999. Diameter of the tetrad consisting of 4 attached pollen grains. This mean excludes clones which produced no tetrads with stained pollen
[u]Significance of differences among crosses was found using analysis of variance on a randomized complete block design.

Fruit production measured during two seasons was highly variable (Table 9), ranging from 1 (score for zero berry production) to 8.5 (9 was the maximum on the scale used). From 47 clones evaluated, 17 (36 %) did not yield any fruit, whereas 11 clones (23 %) were low, 13 clones (28 %) were medium, and 6 clones (13 %) were high fruit producers.

Most clones that produced berries also produced numerous seeds (Table 9) although most of the seeds were not plump and were probably nonviable. Based on 10-berry samples, the average number of seeds per berry for all MIK clones was 24.2, and ranged from 0 to 59 seeds per berry. Many of these seeds were small, wrinkled or flattened. The amount of plump seeds per berry was highly variable between clones,

Table 9. Plant size, berry production and seeds per berry of Vaccinium darrowi x V. arboreum open-pollinated derivatives (MIKs).

Clone	Plant height[z] (cm)	Plant diameter[z,y] (cm)	Fruit production[x] (1-9 scale)	Total seeds per berry[w]	Plump seeds per berry[w]
98-208	173	112	2.0	0.0	0.0
98-209	na[v]	na	1.0	--[t]	--
98-210	158	107	4.0	32.2	13.6
98-211	91	53	1.3	na	na
98-212	na	na	1.0	--	--
98-213	163	119	8.5	20.8	1.3
98-214	173	109	2.3	48.9	4.0
98-515	na	na	1.0	--	--
98-216	132	55	1.3	32.0	9.0
98-217	178	116	1.5	na	na
98-218	71	89	6.0	17.1	2.3
98-219	175	150	5.5	37.0	5.8
98-220	102	38	1.0	--	--
98-221	69	47	1.0	--	--
98-222	69	47	3.8	53.3	8.3
98-224	na	na	1.0	--	--
98-225	132	65	3.0	4.3	1.0
98-226	na	na	1.0	--	--
98-227	na	na	2.8	na	na
98-228	132	86	6.3	23.0	1.5
98-229	168	80	5.8	16.9	5.9
98-230	na	na	1.0	--	--
98-231	97	67	2.3	16.5	1.5
98-232	158	90	1.0	--	--
98-233	na	na	1.0	--	--
98-234	150	51	4.3	12.8	2.8
98-235	na	na	1.0	--	--
98-236	226	104	6.3	23.7	4.9
98-237	196	88	6.0	6.3	1.0
98-238	81	37	1.0	--	--
98-239	175	84	4.3	21.5	7.4
98-240	38	10	1.0	--	--
98-242	160	51	6.0	41.3	2.9
98-243	203	121	6.5	30.3	7.2
98-244	81	52	1.0	--	--
98-245	140	74	5.3	3.8	1.3
98-246	152	91	7.5	19.7	8.3
98-247	198	123	1.0	--[t]	--

Table 9. Continued.

Clone	Plant height[z] (cm)	Plant diameter[z,y] (cm)	Fruit production[x] (1-9 scale)	Total seeds per berry[w]	Plump seeds per berry[w]
98-248	107	60	2.3	na[u]	na
98-249	104	37	1.0	--	--
98-250	165	102	6.0	28.1	11.6
98-251	112	86	4.3	18.7	4.0
98-252	216	69	7.0	56.7	20.9
98-253	104	83	1.3	na	na
98-254	na[v]	na	2.0	19.2	1.1
98-255	173	156	5.5	14.8	2.5
98-256	na	na	1.0	--	--
Probability[s]	--[r]	--	P<0.005)	--	--

[z] Fall, 1999.
[y] Canopy diameter: average of east-west measurement with north-south measurement.
[x] Mean of two years (1998-1999). Scale: 1= no fruit, 9 = full crop.
[w] Summer, 1999.
[v] Plants that died during the evaluation.
[u] Data not available.
[t] Plants that did not yield fruit.
[s] Probability that there were not significant differences among clones in a randomized block analysis of variance with 47 clones and two blocks (years).
[r] One year data collection. Statistical analysis not performed.

ranging from 0 to 20.9. From 25 clones that were evaluated, 4 (16%), averaged one or less plump seeds per berry, 11 clones (44%) averaged 1.5-5 plump seeds per berry, 7 clones (28 %) yielded 5.5-10 plump seeds per berry and 3 clones (12%) yielded more than 10 plump seeds per berry. An additional survey with 5 varieties of southern highbush blueberry that had been open pollinated outside where they could be cross-pollinated by bees ('Star', 'Gulfcoast', 'Jewel', 'Sharpblue' and 'Bluecrisp') shown an average of 33.9 plump seeds per berry (data not shown).

Variables to measure male fertility in the MIK plants are shown in Table 10. Six clones were not evaluated due to lack of flowers. All the remaining clones shed pollen although the amount was variable. Statistical analysis showed significant differences among clones in the amount of pollen shed. Considering only those clones that flowered, 51.2 % were high pollen producers, 29.3 % were moderate producers, and 19.5 % were low pollen producers. Pollen staining with aceto-carmine showed that 7 clones were pollen sterile (Table 10). These clones showed a very low percentage of their pollen stained; the same clones also shed very little pollen. Most of the other MIKs clones had at least 50 % of their pollen stained, but none of the clones was highly male fertile. The maximum percent pollen staining for any MIK clone was 88.7 %. Pollen tetrad size was homogeneous among the clones with an average tetrad diameter of 51.7 microns.

In contrast to the F-1 hybrids, where one out of 4 plants produced unreduced gametes, pollen samples analyzed in the MIK population did not show any unreduced gametes. Apparently, 2n-gamete production in the MIKs is reduced as the influence of the V. darrowi genome is diluted after crossing with other Vaccinium species. On average, all the variables measured indicate that the MIKs were far more fertile than the F-1 hybrids (Table 5). Average fruit yield, number of plump seeds per berry, and pollen amount were increased by 2.7, 26, and 1.6 times, respectively, whereas the percentage of stained pollen was increased from 3.8 % for the F-1s to 60 % for the MIKs.

The analysis of individual plants in the MIK population showed great variability in all variables evaluated except for pollen tetrad diameter. The high variability was expected given the open-pollinated origin of the population. Plants that were near enough to the F-1 plants to have been possible pollen sources during open pollination included

Table 10. Pollen abundance, pollen staining and pollen tetrad size of Vaccinium darrowi x V. arboreum open-pollinated derivatives (MIKs).

Clone	Pollen amount[x] (1-9 scale)[y]	Stainable pollen[x] (%)	Pollen tetrad diameter[xw] (microns)
98-208	6.1	42.9	54.4
98-209	9.0	59.4	52.8
98-210	7.9	79.0	54.0
98-211	6.9	77.2	52.0
98-212	--[u]	--	--
98-213	6.7	41.7	47.5
98-214	6.6	75.0	48.4
98-215	--	--	--
98-216	5.4	74.1	54.2
98-217	7.9	66.7	53.3
98-218	7.7	53.7	49.1
98-219	6.1	81.1	49.9
98-220	3.5	35.9	54.5
98-221	--	--	--
98-222	4.4	74.1	46.2
98-224	2.0	82.8	59.4
98-225	5.8	59.0	49.7
98-226	8.9	80.5	48.6
98-227	6.9	58.8	56.4
98-228	6.1	64.2	51.4
98-229	1.7	10.0	52.3
98-230	1.8	3.2	50.4
98-231	5.8	7.3	46.8
98-232	1.3	15.0	56.9
98-233	1.4	0.0	45.0
98-234	2.4	66.6	54.4
98-235	--	--	--
98-236	8.0	85.5	50.6
98-237	8.5	88.7	51.7
98-238	7.1	83.4	52.6
98-239	7.8	83.4	51.0
98-240	4.6	81.3	52.9
98-242	8.3	45.4	55.4
98-243	6.2	73.5	56.0
98-244	1.3	1.6	48.2
98-245	2.4	23.8	57.4
98-246	7.3	75.8	54.4
98-247	7.7	71.3	58.0
98-248	6.2	72.4	52.2
98-249	--[u]	--	--

Table 10. Continued.

Clone	Pollen amount[x] (1-9 scale)[v]	Stainable pollen[x] (%)	Pollen tetrad diameter[xw] (microns)
98-250	8.7	82.4	54.5
98-251	7.1	76.1	56.2
98-252	5.4	86.1	53.1
98-253	8.3	78.1	53.3
98-254	7.6	87.3	50.0
98-255	6.7	68.4	52.4
98-256	--	--	--
Probability[t]	P<0.01	P<0.005	P(0.63)

[x]Mean of two years (1998-1999). Scale: 1= no pollen, 9= abundant pollen equal to fully-fertile southern highbush blueberry cultivars.
[w]Diameter of four united pollen grains.
[v]Scale: 1= no pollen, 9= abundant pollen equal to fully-fertile southern highbush blueberry cultivars.
[u]Clones that did not yield flowers.
[t]Probability that there were significant differences among clones in a randomized block analysis of variance with 47 clones and two blocks (years).

diploids, tetraploids, and hexaploids. At least potentially, individual MIK plants could have been monoploid, diploid, triploid, tetraploid, pentaploid or aneuploid (Brooks, 1996). The extent to which chromosome number differences contributed to variability in the fertility of the MIK population will be discussed in Chapter 5.

CHAPTER 4
CONTROLLED CROSSES INVOLVING F-1 HYBRIDS, MIK DERIVATIVES, V. corymbosum AND V. ashei.

Introduction

The possibility of using Vaccinium darrowi x V. arboreum F-1 hybrids and the MIK plants that were obtained by open pollinating the F-1 hybrids to introgress desirable traits from V. arboreum into cultivated blueberry needs further study. To test the possibility of obtaining completely-fertile highbush blueberry plants containing genes from V. arboreum, a series of controlled crosses was performed.

A first objective was to determine if fertile progeny could be obtained by hand pollinating V. darrowi x V. arboreum F-1 hybrids with pollen from either V. corymbosum or V. ashei. A second objective was to determine if the F-1 hybrid plants that produced the most unreduced gametes would cross more easily with hexaploid V. ashei or with tetraploid V. corymbosum. Since some microspores appeared as monads in the microscopic pollen survey, it was thought possible that they could be double unreduced gametes (4*x*), and might fuse more successfully with a 3*x* V. ashei gamete than with a 2*x* V. corymbosum gamete.

A third objective was to determine if the selected fertile MIK plants were as male fertile as V. corymbosum cultivars when crossed with V. corymbosum.

Materials and Methods

Crosses were made during Spring 1998 in the greenhouse using selected F-1 hybrids, selected F-1 open-pollinated derivatives (MIKs), V. corymbosum and V. ashei plants. F-1 hybrids were chosen for use as parents after the quantity and quality of pollen produced by 102 F-1 hybrid plants had been evaluated. F-1 plants that produced the highest frequency of monads and diads were selected for crossing with tetraploid southern highbush cultivars. Selected MIKs were chosen for high male fertility. V. corymbosum and V. ashei plants were cultivars or advanced selections from the blueberry breeding program.

Potted plants of V. corymbosum, V. ashei, F-1 hybrids and MIKs were placed in a refrigerator at 5 °C for about 1400 hours during Winter 1997-1998 to satisfy the chilling requirement and to promote synchronous blooming on plants having different chilling requirements. After chilling, the plants were placed in a bee-proof greenhouse where the crosses were made. Thousands of other blueberry crosses had been made in this greenhouse during the previous 10 years and concurrently with excellent results. The crosses made to pursue the first and second objectives are shown in Table11. Crosses made to achieve the third objective are shown in Table 12.

Pollen was obtained from flowers from potted plants of V. corymbosum, V. ashei and MIK plants that were in the greenhouse. Pollen from the F-1 hybrids was obtained from flowering branches collected from selected plants at the Hort Unit and maintained in jars of water.

The F-1 V. darrowi x V. arboreum clone 97-251 was selected for special study because it had produced far more fruit, seed, and viable seedlings than any other F-1

Table 11. V. corymbosum and V. ashei clones used as females, and source of pollen from selected V. darrowi x V. arboreum F-1 hybrids used to pollinate them.

V. corymbosum (4x)		F-1 V. darrowi x V. arboreum hybrid (2x)	V. ashei (6x)		F-1 V. darrowi x V. arboreum hybrid (2x)
95-54	x	98-50	94-216	x	98-50
98-14	x	98-50	96-162	x	98-50
97-27	x	98-139	94-216	x	98-139
95-52	x	98-155	96-162	x	98-155

Table 12. V. corymbosum clones used as females and MIK and F-1 hybrid pollen sources used to pollinate them.

Female clone	Pollen sources	
V. corymbosum	V. corymbosum	V. darrowi x V. arboreum open pollinated (MIK)
Star	A-17	93-90
84-33	E-12	98-183
84-37	91-191	91-333
91-16	91-191	98-182
98-13	Star	95-58
		V. darrowi x V. arboreum F-1 hybrid
95-58	91-183	98-155
97-27	92-236	98-139
98-14	90-4	98-50

open pollinated in the field in 1996. This clone was hand-pollinated in the greenhouse in 1998 to see if it was also unusually fertile in these crosses.

For each attempted cross about two hundred and fifty flowers were pollinated during the bloom period with freshly gathered pollen, which was applied to the stigma with the thumbnail. Afterwards, all non-pollinated flowers were removed from the plants. During the ripening period, berries were harvested and counted twice a week. The number of seeds per berry and berry weight were obtained from 10 and 20 berries, respectively. After harvest was completed, seeds were removed from all the remaining berries using a food blender and were stored dry at 5° C. Data collected for the different crosses were fruit set percentage and total seed weight. During Fall 1998, 0.5 g of seed of each cross was sown on peat pots in a greenhouse under mist for 3 hours per day. The number of seeds that germinated were recorded for each cross. Using data for total seed weight per cross, the number of seedlings per 100 pollinated flowers was estimated for each cross.

For the third objective, V. corymbosum clones were used as female parents (Table 12). For some clones one large potted plant was used. The branches of this plant were divided into two groups. One group was pollinated with pollen from either a MIK or an F-1 hybrid. The other branches were pollinated with pollen from a V. corymbosum clone. For other V. corymbosum clones, two plants derived from softwood cuttings were used as females. One plant was pollinated with MIK or F-1 hybrid pollen, the other with V. corymbosum pollen. For all the crosses, ripe fruits were harvested weekly, and after all the fruit was collected, a fruit set percentage was calculated as the number of ripe fruit harvested divided by the number of flowers pollinated.

Two samples of 20 and 10 fruits were selected at random. The 20-berry samples were weighed, and each berry in the 10-berry sample was opened by hand to determine the seed content. Seeds in the remaining berries from all crosses were extracted with a food blender and were air-dried. After being dried, the seed were stored in paper bags at 4 ° C for 5 months, and then were sown in November on peat moss under intermittent mist in a greenhouse.

The temperature in the greenhouse was allowed to vary between 5 and 30°C. Germination percentage was determined before seedlings were transplanted to the field.

Statistical Analysis

A Chi-square test of independence was used to compare the results of pollinating with different pollen sources (Steel and Torrie, 1960). In testing fruit set percentage, the null hypothesis was that the ratios formed by dividing the number of berries produced by the number of flowers pollinated were equal for the two pollen sources being compared.

For comparing the number of seedlings produced per pollinated flower, the null hypothesis was that the ratio defined by dividing the number of seedlings obtained by the number of flowers pollinated was the same for the two pollen sources being compared. A calculated 1degree of freedom Chi-square that was significantly greater than zero indicated that these ratios differed for the two pollen sources.

A randomized block ANOVA was conducted to determine whether there was significant difference between pollen sources in seed weight per berry. In this analysis, pollen sources were used as treatments and female parent was used as a block.

Results and Discussion

Highbush x F-1 V. darrowi-V. arboreum Crosses

Results from pollinating four clones of highbush blueberry (V. corymbosum, 2n=4x=48) with pollen from four other highbush clones and three V. darrowi x V arboreum F-1 hybrids (2n=2x=24 are shown in Table 13.

In all the highbush clones used as females, the percent of pollinated flowers that produce a mature fruit was lower ($P < 0.005$) when F-1 hybrid pollen was used instead of pollen from the highbush cultivars. Seed weight per berry and fruit set percentage averaged 1.9 mg and 10 %, respectively, in clones pollinated with F-1 hybrids and 16.1 mg and 85 %, respectively, in the same clones pollinated with highbush.

Even though the seed weight per berry and the fruit set were low, all the crosses involving F-1 hybrids as a pollinator gave some viable progeny, but the seedling number was very low compared to the number of seedlings obtained when the same highbush clones were pollinated with highbush pollen. The average number of seedlings produced per 100 pollinated flowers was 5.7 versus 697 when highbush clones were pollinated with F-1 hybrid and highbush pollen, respectively.

The three F-1 hybrid clones used here were chosen because they produced the most monads and diads in the pollen fertility survey (Chapter 3, Table 3). The triploid block is known to be very strong in 4x-2x Vaccinium crosses. Thus, the few viable progeny produced from the crosses between highbush and F-1 hybrids might be expected to be primarily tetraploid, but triploid or even hexaploid progeny might also be possible. Since care was taken to minimize the possibility of self-pollination during pollination of

Table 13. Number of flowers pollinated, fruit set percent, seed weight per berry and number of seedlings produced for controlled crosses in which tetraploid southern highbush clones were the female parents and the pollen parents were either F-1 V. darrowi x V. arboreum hybrids (2n=4x=24) or other southern highbush clones (HB).

Vaccinium corymbosum female parent	Pollen source	Number of flowers pollinated	Fruit set (%)	Seed weight per berry (mg)	Number of seedlings per100 pollinated flowers
95-52	98-155 F-1	275	14.9	0.1	0.7
	91-183 HB	253	83.8	17.4	479.1
			P< 0.005[2]	P< 0.005	P< 0.005
97-27	98-139 F-1	260	9.2	3.3	10.4
	92-236 HB	162	85.8	3.9	10.7
			P<0.005	P>0.05	P>0.05
98-14	98-50 F-1	246	15.0	2.7	10.2
	90-4 HB	127	84.3	29.2	2009.6
			P<0.005	P< 0.005	P<0.005
95-54	98-50 F-1	217	0.9	7.1	1.4
Highbush	Averaged F-1	998	10.4	1.9	5.7
	Averaged HB	542	84.5	16.1	697.7
			P< 0.005	P< 0.005	P< 0.005

[2]Probability that pollen source did not significantly affect the parameter indicated according to the Chi-square test of independence in a 2 x 2 contingency table.

the highbush, the results indicate that V. arboreum genes can be moved into tetraploid southern highbush hybrids using V. darrowi as a genetic bridge.

Rabbiteye x F-1 V. darrowi- V. arboreum Crosses

Crosses using two rabbiteye blueberry clones (V. ashei, 2n=6*x*=72) as females and three F-1 hybrids which had been determined to be 2n-gamete producers as males are shown in Table14. From four crosses made, only one produced berries. The rabbiteye clone 94-216 had 16 % fruit set and 1.2 seedlings per 100 pollinated flowers when it was pollinated by F-1 hybrid clone 98-50. This F-1 hybrid clone was a particularly high producer of unreduced monads and diads in the pollen fertility survey (Table 3).

The F-1 hybrid 97-251, which had been selected for high berry set when open pollinated, gave 12.7 % fruit set when pollinated with pollen from the tetraploid highbush clone 98-86 and 5% fruit set when pollinated by the rabbiteye cultivar Brightwell (Table 15). However, only pollination with highbush pollen produced progeny (1.5 seedlings per 100 flowers). Crossing the F-1 hybrids with hexaploid V. ashei was far less successful than crossing them with tetraploid V. corymbosum.

Highbush x MIK Crosses

Results from crosses between five clones of highbush blueberry (V. corymbosum, 2n=4*x*=48) as female parents and 5 V. darrowi x V. arboreum open-pollinated derivatives (MIKs) and four V. corymbosum clones as pollen parents are shown in Table 16.

The variables evaluated - fruit set, seed weight per berry, and number of seedlings per 100 pollinated flowers - usually did not show statistical differences when V. corymbosum was pollinated by MIK versus highbush pollen. Variation was observed according to the particular clones used in the crosses.

Table 14. Number of flowers pollinated, fruit set, seed weight per berry and number of seedlings for controlled crosses involving Vaccinium ashei (2n=6x=72) as a female parent and V. darrowi x V. arboreum (both 2n=2x=24) F-1 hybrids as a male parent.

V. ashei female parent	F-1 hybrid pollen source	Number of flowers pollinated	Fruit set (%)	Seed weight per berry (mg)	Number of seedlings per100 pollinated flowers[z]
94-216	98-50	250	16.4	0.4	1.2
94-216	98-139	96	0.0	0.0	0.0
96-162	98-50	254	0.0	0.0	0.0
96-162	98-55	239	0.0	0.0	0.0

Number of seedlings per pollinated flower was higher in two cases when highbush was pollinated with highbush, and in one case when highbush was pollinated with MIK pollen. For the other two highbush clones, number of seedlings per pollinated flower did not differ significantly between the two pollen sources.

Even though the number of crosses analyzed was low, the results indicate that V. darrowi x V. arboreum open-pollinated derivatives (MIK) that have been selected for high fertility can be as male fertile as V. corymbosum cultivars when used to pollinate V. corymbosum.

The number of viable progeny produced by highbush when it was pollinated with MIKs was comparable to that produced when highbush was pollinated with highbush. The higher number of progeny from highbush x MIK crosses compared to highbush x V. darrowi-V. arboreum F-1 hybrids is probably due to the homoploid crossing condition (both parents in the highbush x MIK crosses are tetraploid) and the higher

Table 15. Number of flowers pollinated, fruit set percent, seed weight per berry and number of seedlings produced for controlled crosses involving V. darrowi x V. arboreum F-1 hybrids (both parents 2n=2x=24) and MIK derivatives as female parents and Vaccinium corymbosum (HB, 2n=4x=48) and V. ashei (RE, 2n=6x=72) as male parents.

Female parent	Pollen source		Number of flowers pollinated	Fruit set (%)	Seed weight per berry (mg)	Number of seedlings per 100 pollinated flowers
	clone	species				
97-251 F-1	96-86	HB[z]	260	12.7	2.3	1.5
	Brightwell	RE	260	5.0	1.8	0.0
				P[y]< 0.01		0.1<P< 0.25
91-333 MIK	98-20	HB	165	100.0	19.4	24.8

[z]HB = southern highbush blueberry (V. corymbosum hybrids); RE = rabbiteye blueberry (V. ashei).

[y]Probability that fruit set and number of seedlings per pollinated flower did not differ when pollen from the highbush clone was used instead of pollen from the rabbiteye clones according to Chi-square test of independence in a 2 x 2 contingency table.

genome homology between highbush and MIK than between highbush and F-1 hybrids. The proportion of V. arboreum genes is lower in MIKs compared to the F-1 hybrids. The genetic composition of the most fertile MIK clones is believed to be about one quarter V. arboreum, one quarter V. darrowi, and half southern highbush (Lyrene and Brooks, 1995); the genetic composition of the F-1 hybrids is half V. darrowi and half V. arboreum. If unreduced gametes from the F-1 hybrids constituted the maternal contribution to the zygotes in the highbush x F-1 crosses, those zygotes would contain one quarter V. arboreum genes compared with one-eighth of V. arboreum in the zygotes from highbush x MIK crosses.

Table 16. Number of pollinated flowers, fruit set, seed weight per berry and number of seedlings per 100 pollinated flowers in controlled crosses involving highbush (HB) as a female parent and either highbush or MIK as male parent.

Vaccinium corymbosum female parent (HB)	Pollen source	Number of flowers pollinated	Fruit set (%)	Seed weight per berry (mg)	Number of seedlings per100 pollinated flowers[z]
Star	Millenia (HB)	210	96.5	6.2	549.6
	93-90 (MIK)	270	100.0	6.4	474.6
			P< 0.005		P>0.05
84-33	Santa Fe (HB)	294	88.1	7.1	211.9
	98-183 (MIK)	295	87.5	7.0	813.3
			P>0.05		P<0.005
Sapphire	91-191 (HB)	250	90.8	28.2	2113.2
	91-333 (MIK)	257	95.7	21.6	1947.2
			P< 0.05		P>0.05
91-16	91-191 (HB)	250	80.4	16.8	803.8
	98-182 (MIK)	250	75.2	8.3	248.8
			P>0.05		P< 0.005
98-13	Star (HB)	245	100.0	13.5	1493.0
	95-58 (MIK)	260	85.0	9.7	929.9
			P< 0.005		P< 0.005
Highbush	Highbush	1249	91.2	13.7	938.7
	MIK	1332	89.8	11.1	955.5
			P>0.05		P>0.05

[z]Probability that fruit set and number of seedlings per pollinated flower were greater or did not differ when highbush pollen was used instead of pollen from MIKs according to Chi-square test of independence in a 2 x 2 contingency table.

CHAPTER 5
CYTOGENETICS OF V. darrowi x V. arboreum F-1 HYBRIDS AND THEIR OPEN-POLLINATED DERIVATIVES (MIK)

Introduction

An understanding of species genome relationships is useful to plant breeders in their attempts to transfer desirable traits from wild species to related cultivated species. Analysis of pairing behavior during prophase I (PI) and metaphase I (MI), as well as disjunction patterns during anaphase I (AI) and II (AII) and telophase I (TI) and II (TII) is useful to determine the genetic homology between the two genomes in an interspecific cross. Viable hybrids have been obtained between V. darrowi and V. arboreum even though they are classified in different sections of genus Vaccinium (Lyrene, 1991). Both species are diploid. The meiotic behavior of these hybrids is unknown, and the utility of the hybrids as bridges to transfer V. arboreum genes into the cultivated blueberry is still being assessed. The objectives of this study were as follows:

1) To analyze meiosis in V. darrowi x V. arboreum F-1 hybrids and to determine whether there were abnormalities during meiosis that might reduce fertility and affect their usefulness as breeding lines.

2) To study meiosis during microgamete formation in a random MIK population and to determine whether chromosome pairing and disjunction differ for fertile and sterile MIKs.

3) To determine the ploidy level in the MIKs and to see if they produce any aneuploid nuclei during meiosis.

Materials and Methods

To study meiotic behavior in pollen mother cells, flower buds of the appropriate stage of development for meiosis were collected from F-1 V. darrowi x V. arboreum hybrids and F-1 open-pollinated derivatives (MIKs) of varying fertility levels (sterile to high fertile). The flower buds were collected from January to March in 1999 and 2000. The samples were fixed in a 3:1 mix of glacial acetic acid : absolute ethanol and were stored at 5° C. The fixative was replaced three times during the first 10 days to remove pigments. For long-term storage, the fixed buds were transferred to 70% ethanol and put in a refrigerator at -18° C. Prior to examination, the fixative was removed by soaking the flower buds in water for five minutes. As meiotic flower buds are difficult to find, and only a small percentage of the flower buds collected on any particular day contain meiotic cells, flower buds were examined microscopically previous to enzymatic treatment. Both F-1 hybrids and MIK derivatives produce flowers in corymbs that originate from axillary and terminal buds. When each corymb bud begins to grow, it produces a short stem, with 5 to 10 flowers arranged alternatively along this stem. The first flowers that undergo meiosis are those at the base of the stem. The last ones to undergo meiosis are at the tip. In searching for meiosis, one anther was removed at random from one flower to see if meiocytes were present. Meiocytes were easy to recognize because they were contained in a unique undivided sac whose characteristic appearance made them immediately apparent when an anther was macerated in 45 % acetic acid and viewed at 250-x magnification with a phase-contrast microscope. If meiosis had already occurred, the sac will have developed compartments to form a typical tetrad pollen grain.

Meiosis occurs in cells that have ceased to propagate mitotically and have developed an envelope or sac, which retains a distinctive appearance until the sac divides to form a spore tetrad. If the sampled anther contained meiocytes, the other anthers from that flower were transferred to a digestion fluid for 12 hours at 50° C for protoplast isolation. The digestion fluid was made by placing 0.02 g pectinase and 0.02 g cellulysine (Karlan Research Products) in 3 ml of citrate buffer solution at pH 5.0. After digestion, anthers were washed in distilled water and could be stored in 70 % ethanol for later viewing if needed. Each anther was used to prepare one slide. The washed anther was placed directly on a slide with a small drop of 45% acetic acid. Then the anther was cut into 3 to 4 small pieces with a scalpel and spread over an area on the slide approximately 3 x 3 mm to avoid tissue overlapping. Then a cover slip was added and it was gently tapped with a pencil to spread the cells. More 45% acetic acid was then added. The slide was heated gently over a flame for one second and allowed to cool. Heating and cooling were done three times. After the third cooling, the cover slip was gently pressed with a paper towel between the slide and the thumb and was sealed with wax. A Leitz phase contrast light microscope was used to observe the chromosomes and to make microphotographs at 250x and 1000x magnification. Observations were collected from meiocytes from anthers from the same or different flowers from the same plant.

To answer the question of whether the V. arboreum chromosomes and the V. darrowi chromosomes in the F-1 hybrids and F-1 open-pollinated derivatives (MIKs) are sufficiently homologous to pair during PI and MI of meiosis, the average number of univalents, bivalents, trivalents and other associations at metaphase I were recorded for six F-1 hybrids and eight MIK clones. In addition, meiotic cells were studied to evaluate

whether normal AI and normal AII were giving rise to four spores, each with the haploid chromosome number. Cells were recorded in which first division restitution occurred, followed by a normal second division separation, giving rise to two spores, each with the double chromosome number (2n-gametes). Also during AI and AII, lagging chromosomes or excluded chromosomes were observed and recorded. Finally, production of aneuploid spores was noted. Meiotic irregularities would indicate that abnormal meiosis contributed to reduce pollen fertility in these hybrids.

Results and Discussion

Cytogenetics of V. darrowi and V. arboreum

Data on meiotic chromosome associations for parental V. darrowi are presented in Table 17. Meiocytes for V. arboreum could not be found in the samples that had been collected.

Meiosis was normal in V. darrowi species. It invariably showed twelve independent bivalents at MI (Fig.1). Chromosomes in V. darrowi typically produced ring bivalents (formed by two distal chiasmata) or rectangular bivalents with none, one or two openings. Only independent bivalents and no secondary associations were observed at the end of PI and MI. Chromosome counts at AI confirmed that the V. darrowi genotypes studied are diploid ($2n=2x=24$). Disjunction of the chromosomes at AI was observed to be regular (Fig. 2). At AII, the great majority of the nuclei revealed regular disjunction of the chromosomes. No abnormalities were observed in the different stages of meiosis, and numerically balanced gametes were produced.

Table 17. Distribution of types of chromosome associations [univalents (I), bivalents (II), trivalents (III) and quadrivalents (IV)] at M I in diploids V.darrowi (2n=2*x*=24), V. arboreum (2n=2*x*=24), F-1 hybrids (2n=2*x*=24) and tetraploid and pentaploid MIK derivatives (2n=4*x*=48 and 2n=5*x*=60).

Taxa	% pollen stainability	Number of cells		I	II	III	IV
V. darrowi	71.1	40	Mean	0	12	0	0
			Range				
F-1 hybrids	3.8	134	Mean	16.32	3.99	0	0
			Range	8-24	0-8		
MIK (4*x*)	60.3	59	Mean	8.13	18.19	0.02	0.85
			Range	0-18	13-23	0-1	0-4
MIK (5*x*)	10.0	5	Mean	28.6	11.8	0.2	1.8
			Range	15-48	6-17	0-1	0-4

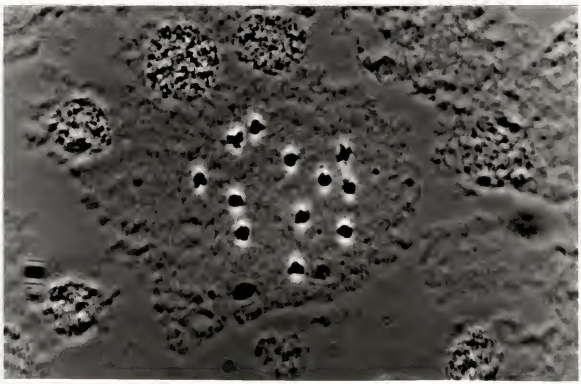

Figure 1. Late diakinesis in V. darrowi showing 12 II.

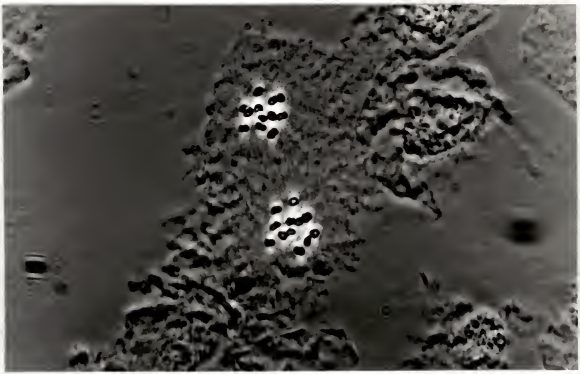

Figure 2. A I in V. darrowi showing 12:12 separation.

The flower bud samples collected from V. arboreum allowed only second meiotic divisions to be observed. AII and TII were normal with even disjunction and 12 chromatids in each nucleus. No abnormalities were observed.

Cytogenetics of the F-1 V. darrowi x V. arboreum Hybrids

Meiosis in the F-1 hybrids was characterized by incomplete pairing, which was variable within and between hybrids plants (Table 18). Many of the hybrid pollen mother cells had a high proportion of univalents at MI (Table 17, Fig. 3 and 4).

Few chromosomes showed normal pairing (Fig. 5). Polar views of MI plates gave an average of 16.32 univalents and 3.99 bivalents. F-1 hybrids showed various irregularities at the first and second meiotic divisions (Tables 19 to 22).

Table 18. Distribution of types of chromosome associations [univalents (I), bivalents (II), trivalents (III) and quadrivalents (IV)] at M I in diploid V. darrowi x V. arboreum F-1 hybrids (2n=2x=24).

Clone	Pollen stainability (%)	Number of meiocytes		I	II	III	IV
98-50	26.2	27	Mean	17.5	3.3	0	0
			Range	8-24	0-8		
98-53	0.2	28	Mean	17.5	3.2	0	0
			Range	14-20	2-5		
98-67	0.0	18	Mean	6.4	8.8	0	0
			Range	4-10	7-10		
98- 125	1.0	16	Mean	20.9	1.6	0	0
			Range	18-24	0-3		
98-136	0.0	2	Mean	23.0	0.5	0	0
			Range	22-24	0-1		

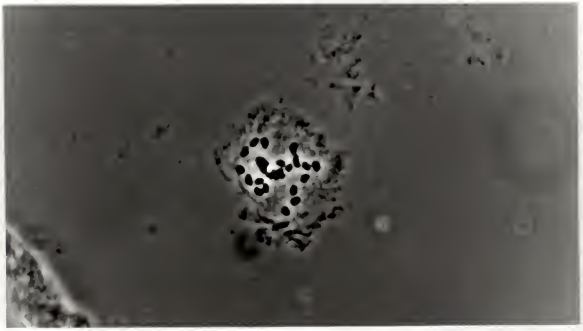

Figure 3. M I in F-1. V. darrowi x V. arboreum hybrid, clone 98-50, showing 18 I + 3 II

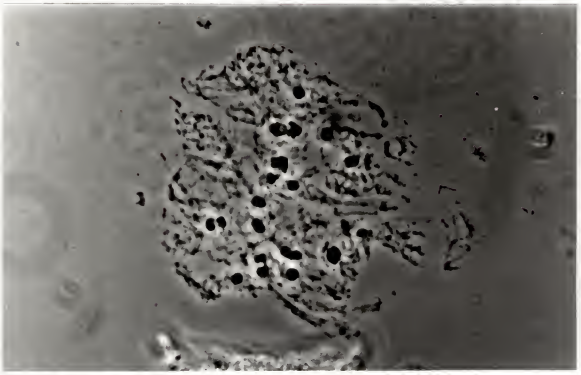

Figure 4. MI in F-1 V. darrowi x V. arboreum hybrid, clone 98-50, showing 14 I + 5 II

Table 19. Balanced (normal) and unbalanced nuclei at first meiotic division in pollen mother cells (PMC) of V. darrowi x V. arboreum F-1 hybrids.

Clone	Pollen stainability (%)	Number of PMC observed	Balanced division	Unbalanced division: With lagging chromosomes and/or bridges	Unbalanced division: Without lagging chromosomes and/or bridges
98-53	0.2	33	13	20	0
98-67	0.0	85	55	25	5

Table 20. Frequency of PMC with lagging chromosomes, chromatid bridges and unbalanced nuclei at first meiotic division in V. darrowi x V. arboreum F-1 hybrids.

Clone	Frequency of PMC N°	Frequency of PMC %	N° of lagging chromosomes	N° of chromatid bridges
98-53	3	9.1	1	0
	2	6.1	2	0
	1	3.0	3	0
	3	9.1	0	1
	5	15.1	0	2
	2	6.1	0	3
	1	3.0	1	1
	1	3.0	1	2
	1	3.0	2	1
	1	3.0	2	2
98-67	7	8.2	1	0
	10	12.4	2	0
	2	11.8	3	0
	3	3.5	1	1
	1	1.2	2	1
	1	1.2	3	1
	1	1.2	4	2

Table 21. Balanced (normal) and unbalanced nuclei at second meiotic division in V. darrowi x V. arboreum F-1 hybrids.

Clone	Pollen Stainability (%)	Number of PMC observed	Balanced division	Unbalanced division: With lagging chromosomes and/or bridges	Unbalanced division: Without lagging chromosomes and/or bridges
98-67	0.0	15	7	6	2

Table 22. Frequency of lagging chromosomes and chromatid bridges at second meiotic division in V. darrowi x V. arboreum F-1 hybrids.

Clone	Frequency of PMC		N° of lagging chromosomes	N° of chromatid bridges
	N°	%		
98-67	1	6.7	2	0
	2	13.3	3	0
	1	6.7	1	1
	1	6.7	2	1
	1	6.7	3	1

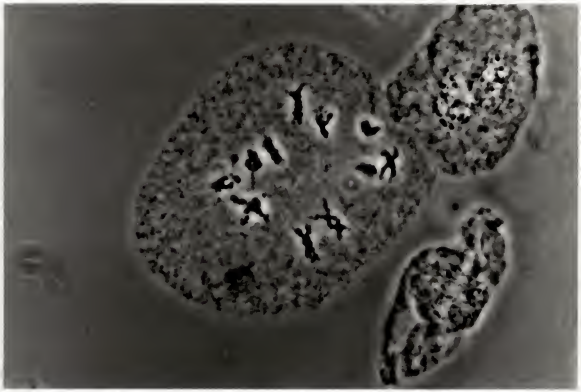

Figure 5. Diakinesis in F-1 V. darrowi x V. arboreum hybrid, clone 98-67, showing chromosome pairing.

Lagging chromosomes were observed at late AI (Fig. 6), probably a result of the high number of univalents at MI. Some of these univalents were afterward lost or formed micronuclei. Lagging chromosomes were also observed at late AII. Chromatin bridges were observed at both AI and AII (Fig 7). Lagging chromosomes and chromatin bridges occurred alone or in combination in the cells observed. When they were in combination, it was not possible to differentiate between lagging chromosomes and chromosome fragments. As a result of abnormalities during disjunction in both divisions, the tetrads formed began to abort soon after meiosis had finished (Fig. 8).

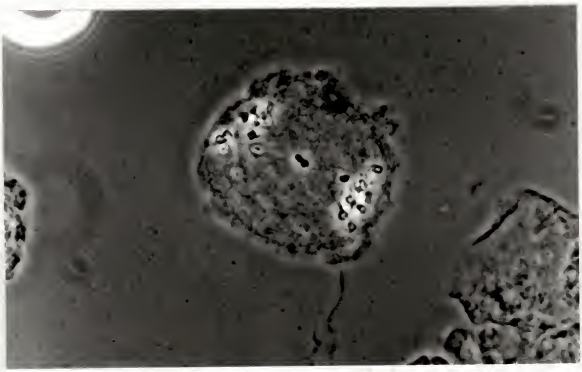

Figure 6. Late A I in F-1 V. darrowi x V. arboreum hybrid, clone 98-67, showing lagging chromosome.

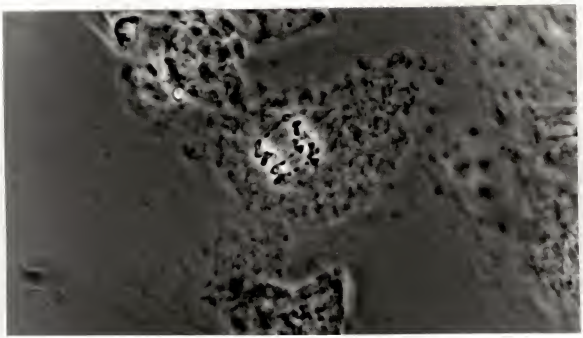

Figure 7. Late A I in F-1 V. darrowi x V. arboreum hybrid, clone 98-53 showing chromatin bridges

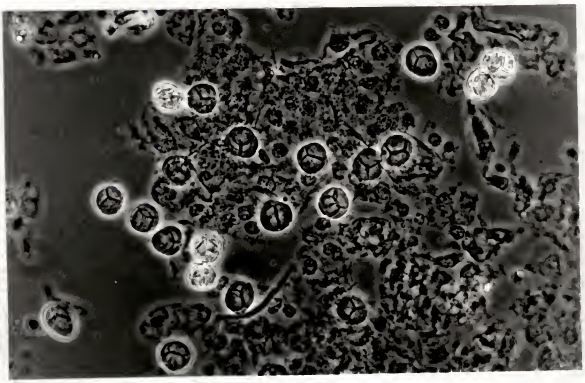

Figure 8. Post-meiosis in F-1 V. darrowi x V. arboreum hybrid clone 98-120, showing tetrads starting to abort.

The degree of relationship between V. darrowi and V. arboreum is indicated in part by the fact that they can easily be crossed to produce vigorous, viable progeny. The low female and male fertility of the F-1 hybrids could be explained at least in part by the low degree of chromosome pairing during meiosis. The two species appear to differ in a paracentric inversion, as is evident from the frequent occurrence of chromatin bridges during the two meiotic divisions. The geographical distribution of V. darrowi and V. arboreum overlap today in north Florida, but it is possible that during the course of evolution, V. darrowi and V. arboreum were geographically separated from each other, and over time become different chromosomally.

Cytogenetics of the F-1 V. darrowi x V. arboreum Open-Pollinated Derivatives (MIK)

The frequencies of various meiotic chromosome associations for F-1 V. darrowi x V. arboreum open-pollinated derivatives (MIK) are presented in Table 23.

Chromosome counts in pollen mother cells during normal disjunction at AI confirmed that 7 of the 8 MIK genotypes studied were tetraploid (2n=4x=48). One genotype was pentaploid (2n=5x=60). Chromosomes of MIK derivatives were generally contracted and clumped at MI (Fig 9) and were difficult to analyze; hence, few clear metaphase plates were recorded. On average, chromosome pairing was increased compared to what was seen in the F-1 hybrids, and multivalent configurations appeared in the MIKs (Fig 10). The average chromosome configuration for the tetraploid genotypes was 8.13 univalents, 18.19 bivalents, 0.02 trivalents and 0.85 quadrivalents, whereas the pentaploid genotype, showed an average of 28.6 univalents, 11.8 bivalents, 0.2 trivalents, and 1.8 quadrivalents (Table 17).

Table 23. Distribution of types of chromosome associations [univalents (I), bivalents (II), trivalents (III) and quadrivalents (IV)] at M I in F-1 V. darrowi x V. arboreum derivatives (MIK).

Clone	Pollen stainability (%)	Number of meiocytes		I	II	III	IV
98-213	41.7	28	Mean	9.96	17.86	0.03	0.54
			Range	4-18	13-21	0-1	0-2
98-218	53.7	7	Mean	9.4	17.6	0	0.8
			Range	4-12	16-20		0-1
98- 229	10.0	5	Mean	28.6	11.8	0.2	1.8
			Range	15-48	6-17	0-1	0-4
98-242	45.4	10	Mean	8.4	18.2	0	0.8
			Range	4-12	16-20		0-2
98-252	86.1	9	Mean	0.25	19.88	0	2.0
			Range	0-2	16-23		1-4

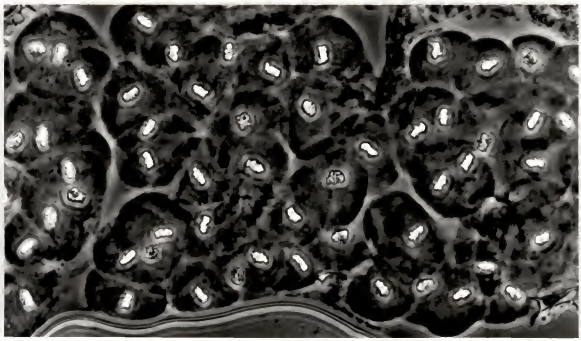

Figure 9. Clumped chromosomes at M I in V. darrowi x V. arboreum open-pollinated derivatives (MIK)

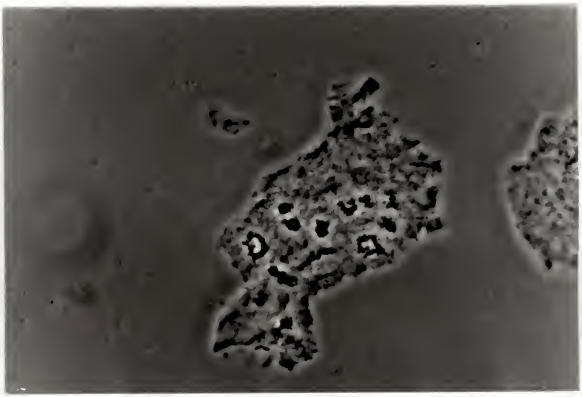

Fig. 10. Bivalents and multivalents (rings and chains) during late diakinesis in V. darrowi x V. arboreum open-pollinated (MIK).

Probably because of better chromosome pairing in the MIKs, the frequency of normal disjunction in the first and second division was increased (Fig 11). However, the degree of chromosome pairing was variable and was correlated with the pollen fertility of the clone (Table 23). The lower the pollen fertility the higher the number of univalents observed at MI.

As in F-1 hybrids, lagging chromosomes (Fig 12), chromatin bridges and unbalanced nuclei (Fig 13) were observed at both divisions in MIK derivative clones.

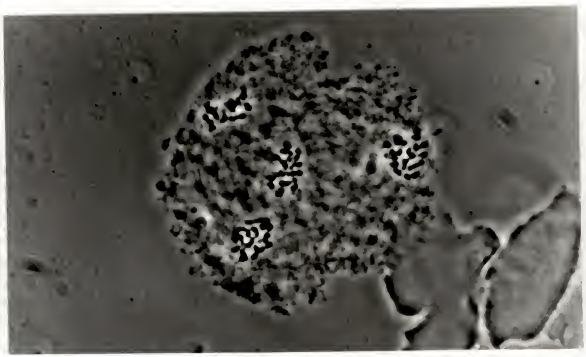

Figure 11. Late A II in V.darrowi x V. arboreum open-pollinated (MIK).

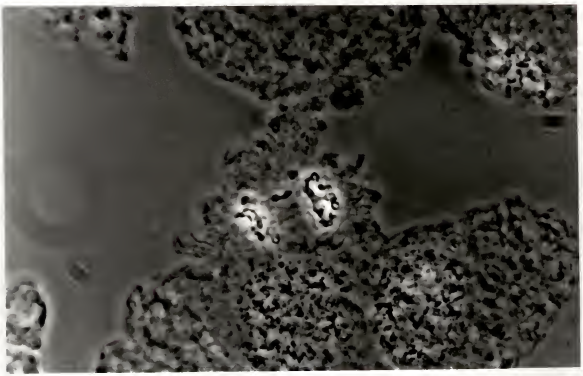

Figure 12. Lagging chromosome at late A I in V.darrowi x V. arboreum open-pollinated (MIK).

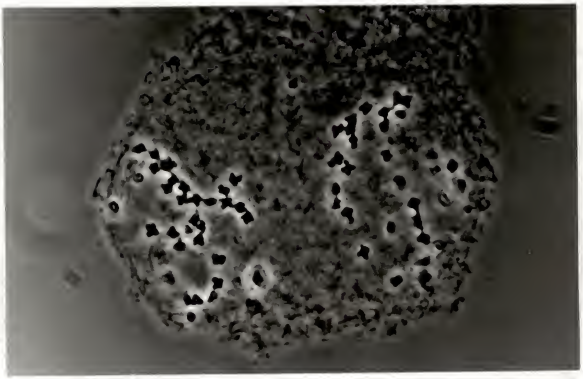

Figure 13. A I with unbalanced nuclei (28/32 separation) in V.darrowi x V. arboreum open-pollinated (MIK)

In an analysis of meiocytes from 8 MIK clones, 26.7 % of the meiocytes observed at A I had lagging chromosomes, 5.4 % had 1-4 chromatin bridges and 2.2% had a combination of both (Table 24). During the second division, the proportions of the same abnormalities observed were 23.3, 0.7 and 2.1 %, respectively (Table 25). Abnormal nuclei, other than lagging chromosomes and chromatin bridges, accounted for 5.4% of the nuclei viewed in the first division (Table 26), where the only abnormality seen, other than bridges and fragments, was aneuploid nuclei. In the second division (Table 27), 28.8% of the dividing cells that were observed either had aneuploid nuclei, micronuclei, six-nucleated meiocytes, or second-division-restitution (Fig. 14).

Table 24. Frequency of lagging chromosomes, chromatid bridges and unbalanced nuclei at first meiotic division in F-1 V. darrowi x V. arboreum derivatives (MIK).

Clone	PMC observed		Number of lagging chromosomes	Number of chromatid bridges
	Number	%		
98-208	1	2.8	1	0
	7	19.4	2	0
	3	8.3	3	0
	1	2.8	0	1
	1	2.8	0	2
	2	5.6	0	3
98-213	1	4.5	1	0
	6	27.3	2	0
	1	4.5	3	0
98-218	2	7.1	1	0
	3	10.7	2	0
	1	3.6	3	0
98-229	7	14.0	1	0
	6	12.0	2	0
	3	6.0	3	0
	1	2.0	4	0
	2	4.0	0	1
	2	4.0	0	4
	3	6.0	1	1
	1	2.0	3	2
98-237	1	20.0	1	0
	1	20.0	0	4
	1	20.0	0	5
98-242	1	9.1	1	0
	1	9.1	2	0
98-252	1	3.3	1	0
	1	3.3	3	0

Table 25. Frequency of lagging chromosomes and chromatid bridges at second meiotic division in F-1 V. darrowi x V. arboreum derivatives (MIK).

Clone	PMC observed		Number of lagging chromosomes	Number of chromatid bridges
	Number	%		
98-213	1	25.0	2	1
98-218	2	6.1	1	0
	3	9.1	2	0
	2	6.1	3	0
	2	6.1	4	0
	1	3.0	5	0
98-229	1	2.6	1	0
	1	2.6	2	0
	5	12.8	3	0
	2	5.1	4	0
	1	2.6	0	3
	1	2.6	2	2
	1	2.6	3	3
98-242	5	8.2	1	0
	2	3.3	2	0
	6	9.8	3	0
	2	3.3	4	0

Both F-1 hybrids and MIKs clones showed chromatin bridges at A I and II. According to Burnham (1962) and Palmer et al., (2000) crossing over within paracentric loops alone or with a crossover in the interstitial segment leads to the appearance of chromatin bridges and fragments at A I and II, and subsequently to aborted spores. Therefore, the observation of bridges at both meiotic anaphases in F-1 hybrids and MIKs is evidence for chromosome pairing with a paracentric inversion.

Table 26. Normal-balanced and unbalanced nuclei at first meiotic division in F-1 V. darrowi x V. arboreum open pollinated derivatives (MIKs).

Clone	Pollen Stainability (%)	Number of PMC observed	Balanced division	Unbalanced division: With lagging chromosomes and/or bridges	Unbalanced division: Without lagging chromosomes and/or bridges
98-208	42.9	36	21	15	0
98-213	41.7	22	14	8	0
98-218	53.7	28	22	6	0
98-229	10.0	50	15	25	10
98-236	85.5	2	2	0	0
98-237	88.7	5	2	3	0
98-242	45.4	11	9	2	0
98-252	86.1	30	28	2	0

Table 27. Balanced (normal) and unbalanced nuclei at second meiotic division in F-1 V. darrowi x V. arboreum derivatives (MIKs).

Clone	Pollen stainability (%)	Number of PMC observed	Balanced division	Unbalanced division: With lagging chromosomes and/or bridges	Unbalanced division: Without lagging chromosomes and/or bridges
98-213	41.7	4	3	1	0
98-218	53.7	33	18	10	5
98-229	10.0	39	9	12	18
98-242	45.4	61	27	15	19
98-252	86.1	9	9	0	0

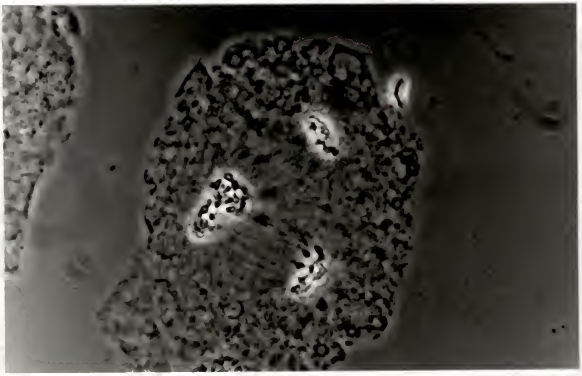

Figure 14. T II in V. darrowi x V. arboreum open-pollinated (MIK), clone 98-229 showing second-division restitution.

The proportion of abnormalities observed was higher in the pentaploid clone, where 67% and 78 % of the meiocytes observed at first and second division, respectively, were abnormal. In the tetraploid MIKs the proportion of abnormalities were 29 % and 47 %, respectively.

Tetraploid clones probably originated by the union of an unreduced egg (2*x*) from a F-1 hybrid, and a normal reduced pollen grain (2*x*) from the abundant population of tetraploid southern highbush blueberry (2n=4*x*=48) growing near the F-1 hybrids clones.

The pentaploid clone (98-229) was most likely formed when normal pollen (3*x*) from V. ashei (2n=6*x*=72) fertilized an unreduced egg (2*x*) of an F-1 hybrid. Another possibility is that a double-unreduced egg (4*x*) from an F-1 hybrid was fertilized by a normal reduced gamete (1*x*) from diploid species such as V. darrowi, V. elliottii, or F-1 V. darrowi x V. arboreum hybrids. Because doubled-unreduced pollen was observed frequently as monads in the fertility survey of F-1 hybrid population, it is also possible that 4*x* eggs can be formed by the F-1 hybrids during megasporogenesis.

It was intended as part of the study of meiosis in the MIK population to sample a few of the most fertile plants and a few of the least fertile plants to see how they differed. It turned out to be much harder to find meiotic cells in the plants that had the lowest fertility. This suggests that low fertility in some cases was due to factors acting before meiosis and was not due entirely to meiotic irregularities.

CHAPTER 6
CONCLUSIONS

One hundred and ten F-1 intersectional hybrids that originated from seven controlled crosses between 4 genotypes of V. darrowi (section Cyanococcus, 2n=2*x*=24) and 4 genotypes of V. arboreum (section Batodendron, 2n=2*x*=24) showed very low female and male fertility as demonstrated by very low pollen shed, low pollen staining and very low numbers of fruit per plant and plump seeds per berry. According to cytogenetic analysis, the hybrids showed low chromosome pairing, and this was probably a primary reason for the low fertility. The large number of univalents at metaphase I in the hybrids indicates low or partial homology between the genomes of the two parent species. Despite low or poor genome homology, the plants were not totally sterile; they produced some stained pollen and viable open-pollinated seed.

The F-1 progeny from the seven V. darrowi x V. arboreum crosses did not differ significantly in mean values of the female and male fertility parameters evaluated. Progeny from the different crosses varied in plant vigor.

Some V. darrowi plants and F-1 V. darrowi x V. arboreum hybrids produced occasional unreduced gametes, which allowed introgression of F-1 hybrid genes into tetraploid V. corymbosum. Controlled (hand) crosses between V. corymbosum and particular F-1 hybrids that produced unreduced gametes produced few viable progeny, but they did show that V. arboreum genes can be moved into tetraploid southern highbush hybrids using V. darrowi as a genetic bridge.

Hand crosses between hexaploid V. ashei and F-1 V. darrowi x V. arboreum hybrids that produce unreduced gametes gave no viable progeny. The low fertility of the hybrids and the low degree of the chromosome pairing indicate that V. darrowi and V. arboreum are not closely related in spite of the fact that F-1 hybrids between the two species can be easily produced by hand pollination in a greenhouse. In addition to genetic barriers to gene exchange, another factor that isolates the two species where their native ranges overlap is flowering time; V. darrowi is normally finished flowering before V. arboreum begins.

Average female and male fertility, although highly variable, were much higher in the F-1 V. darrowi x V. arboreum derivatives (MIKs) than in their F-1 parents. The MIKs also had much higher chromosome pairing than the F-1 hybrids. The higher ploidy level of the MIKs (tetraploid) and the relative increase in the proportion of section Cyanococcus chromosomes apparently improved the chromosome pairing and resulted in the production of a high number of viable gametes. MIKs that had been selected for high fertility were as male fertile as V. corymbosum cultivars when used to pollinate V. corymbosum.

Cytogenetic study of those MIKs that had low fertility revealed reduced chromosome pairing and abnormalities during the first and second meiotic divisions that produced unbalanced and/or aborted pollen.

Both the diploid F-1 hybrids and the low-fertility MIKs had a high frequency of lagging chromosomes, which lead to unbalanced gametes and the formation of micronuclei. A feature that showed chromosome structural differences between sections Batodendron and Cyanococcus was the frequency of 1 to 4 chromatin bridges during first

and (less frequently) second meiotic divisions, which were probably the result of paracentric inversions in the structure of homoeologous chromosomes. These chromatin bridges persist in both meiotic divisions of the MIK derivatives. The frequency of abnormalities during meiosis was highest in the least fertile MIKs.

One 2n=5*x*=60 progeny was obtained by open-pollination of a diploid F-1 hybrid in the field. The most likely origin of this pentaploid is union of a 2n-gamete from the diploid with a 3x gamete from the hexaploid V. ashei.

The results presented in this study demonstrate that gene transfer between V. arboreum and Cyanococcus species is feasible and the tertiary gene pool, represented by Vaccinium species outside section Cyanococcus, could contribute to the production of blueberry cultivars with a wider range of characteristics.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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